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EDITOR'S VIEWPOINT

Green, Dodd, and Iverso (p. 331), in a further study of the flora of caries-immune individuals, developed a method for dispersing dentobacterial plaque material with ultrasonic energy and investigated the bacteria of such plaques. Lactobacilli were low in numbers, or absent, in typical immunes. The plaque did not always mirror the saliva in its flora. The study of bacteria of the plaque is as important, or more important, than that of saliva. This method offers opportunity for more complete observations.

The lipolytic and esterolytic activity of saliva and 16 oral bacteria was investigated by Mahler and Chauncey (p. 338). Different activity was demonstrated in different bacteria, true lipase activity being observed only in one strain of *M. pyogenes,* while saliva, 3 lactobacillus species and some cocci showed overlapping of enzymatic activity.

Turner, Anders, and Becker (p. 343) added thiamine to saliva-corn starch mixtures and compared the dextrinizing time with saliva from the same subjects without added thiamine. The addition of thiamine speeded dextrinizing time and the acceleration was greater in a caries-resistant group than in a caries-prone group. The possibility that the lower pH (from added thiamine Cl-HCl) may be producing this effect must be considered.

Shaw and Sweeney (p. 349) present the results of feeding various antibiotics to intact and sialo-adrenalectomized white rats and cotton rats and of screening tests of antibiotics against two strains of oral lactobacilli. Their results are most interesting, showing species differences in caries inhibition by various antibiotics and differences between in vivo and in vitro results. Penicillin, an antibiotic of limited spectrum, was more effective in reducing caries activity in vivo than broad spectrum antibiotics. These paradoxical findings emphasize the multiplicity of unknowns in the bacteriology of dental caries.

Williams and Franck (p. 361) undertook a study of the antigenic properties of heterofermentative oral lactobacilli. From five strains, six major antigens were observed as well as two minor antigens. On the basis of this study, the authors propose retaining morphologic and physiologic characteristics of these lactobacilli to distinguish between strains because definition into specific serologic groups would be premature.

The problem of acquired caries resistance in hamster colonies once quite susceptible to caries when placed on cariogenic diets has been perplexing and annoying. While many methods have been utilized in efforts to increase caries resistance in experimental animals, few have attempted to increase caries susceptibility. Mitchell and Johnson (p. 372), utilizing a carbonic anhydrase inhibitor and diuretic, claimed to have decreased salivary flow and buffer capacity of human saliva, increased caries activity in hamsters which had become resistant. It seems unquestionable that caries resistance may be decreased because of something which is present in the host as well as being increased by some factor in the host's oral environment.

Cohen (p. 375) took three salivary samples from each of 1,049 Naval recruits (17.5 years of age) and compared the Snyder colorimetric test and salivary lactobacillus index (Rogosa medium) with each other and with the DMF under rather exacting conditions of saliva collection. Placing the two tests into five categories each, there was a high correlation between the Snyder colorimetric test and caries experience, between lactobacillus counts on synthetic medium and caries experience and between the Snyder and lactobacillus tests. The results of correlation with DMF are most interesting since the DMF records only past experience and the salivary tests are designed to measure caries activity. Perhaps the similarity of diet and of living conditions of the recruits, as well as the

rigid conditions of collection of samples, contributes to the reproducibility and correlation of data.

Muhler, Bixler, and Shafer (p. 382) have confirmed that thiouracil and I^{131} are cariogenic for the rat and that desiccated thyroid is anticariogenic for the rat. The co-administration of thiouracil and I^{131} indicates a synergistic effect of these two agents in producing dental caries. While fluoride administered in water has reduced the caries in animals receiving the cariogenic drugs, no synergism was noticed when thyroid and fluoride were fed together.

Analysis of rat carcasses, their fluoride intake and their fluoride excretion by Weddle and Muhler (p. 386) indicates that there is no essential difference in fluoride metabolism whether sodium fluoride, sodium silicofluoride, or stannous fluoride is added to their drinking water at 1 ppm. However, calcium chloride appears to have an inhibiting action on fluoride retention within the skeleton as well as on its absorption.

McCann and Bullock (p. 391) analyzed bone, dentin, and enamel of rats given water with 100 ppm of sodium fluoride for 100 days after weaning. The fluoride levels of the tissues increased as did the magnesium levels but not calcium levels. The fluoride level of tissues from incisors (teeth of persistent growth) was higher than that of the molars (teeth of limited growth). The authors propose that their findings support the theory that the in vivo reaction of crystals of bone, enamel and dentin, with fluoride ions is the formation of fluorapatite.

Muhler (p. 399), who previously reported significant reduction in dental caries with a specially compounded stannous fluoride-containing dentifrice, tested the efficacy of a dentifrice containing stannous fluoride and dicalcium phosphate. In this latter instance, no significant reduction in dental caries was found after six months and one year of unsupervised use. When both stannous fluoride and stannous gluconate were added to a dentifrice containing heat-treated calcium orthophosphate, an ingredient of the effective stannous fluoride dentifrice, no significant reduction in dental caries was found. This study is most important for it indicates that not only is the fluoride compound, or any other anticaries agent added to a dentifrice, critical but also that the polishing agent and all other ingredients in a dentifrice must be considered. This most important finding must be given full consideration when the value of a mixture of ingredients is being considered rather than the value of a single ingredient of that mixture.

Studies on salivary glands of hypophysectomized and control rats given hormonal supplements support the thesis that the salivary glands of rats are influenced by pituitary and thyroid hormones (Clark, Shafer, and Muhler, p. 403). Desiccated thyroid stimulates the salivary glands only in the presence of the pituitary gland. Thyroxin and testosterone, given together, failed to restore salivary gland activity in the absence of the pituitary. The active pituitary factor has not been identified.

Bhussry and Bibby (p. 409) systemically studied the changes with aging at the surface of the enamel. The brown pigmentation of the enamel surface was found to increase with age in accordance with the clinical observation of darkening of enamel with age. These pigmented areas have increased resistance to decalcification, in vitro. It is suggested that they are organic in nature and increase resistance to decay. These observations are in accord with those of Enright, Tresher, and Friesell (J. D. Res. 12: 759, 1932) that "arrested caries" is more resistant to decalcification due to increased organic content and supports the suggestion of Gore that decalcified areas be treated by application of celloidin.

Hansen and English (p. 417) irradiated the incisors of rats and sacrificed them at various periods of 24 hours to 21 days after treatment. Histologic studies showed marked changes in the young odontogenic cells although the radiation was not sufficient to destroy more mature cells. A "second tooth" was observed to arise from the surviving undifferentiated cells. This report of marked deformation of growing teeth of rats subjected to irradiation should in no way be considered indicative of danger to mature teeth from radiation used in diagnosis of dental disease. Unfortunately, such observations in related areas have led to unwarranted interpretations in the clinical field.

Auskaps and Shaw (p. 432) describe the dentition of the small Old World cynomolgus monkey. A very large number of the teeth

examined in seventeen skulls showed pit and fissure caries and two smooth surface lesions of caries were observed. It is suggested that these animals may become of value in experimental studies of dental caries.

Kalnins (p. 437) applied pressure to exposed pulps by means of a calcium hydroxide-sulfathiazole paste. Histologic study of 48 teeth indicated that compacting of the superficial portion of the pulp, with no disturbance of deeper tissues and without prevention of "bridging," resulted when pressure was applied. The author suggests that pressure may favor pulp healing by control of hemorrhage. These findings, which are contrary to assumptions usually applied in clinical treatment, should be carefully evaluated by continued and more extensive clinical and laboratory studies.

Fisher, Shumacher, Robinson, and Sharbondy (p. 447) re-investigated the oxygen consumption of bovine dental pulp by the Warburg method. Substituting air for oxygen as the gas phase resulted in considerable variation in oxygen consumption. They suggest that this is due to "physiologic peculiarities . . . that, at present, lack adequate explanation."

Berliner (p. 451) investigated the x-ray density of mandibular bone as a guide to diagnosis and prognosis of periodontal disease. He concludes that, since x-ray density is itself a variable with bone loss, a clinical diagnosis and prognosis based on radiograph examination alone, is unreliable.

Wilson, Phillips, and Norman (p. 458) found little or no difference in the residual mercury content of amalgam condensed by different methods. The residual mercury was slightly higher with the "increasing dryness" technic in comparison with the technic of wringing out the entire mass. The mercury content at the margins was consistently higher than at the body. Of course, these comparable results presume equal skill and thoroughness applied with each method.

Castaldi, Phillips, and Clark (p. 462) evaluated the effect of various technics on the contour of Class II restorations. As with hand condensation, they found that wedging the matrix band is essential in preventing gingival overhang even when mechanical condensers are used. While "flossing" the restoration immediately after removing the matrix band did improve the contour and reduce overhang, it was of little value after 4 minutes and at no time equal to wedging the matrix. No technic was superior in reducing the higher mercury content of proximal and gingival areas. This type of study contributes to clinical dentistry by substituting the experimental method for empiricism.

Swartz, Norman, Gilmore, and Phillips (p. 472) measured loss and gain in water by hydrocolloid impression material under different conditions. While there was variation between brands, in general, the least amount of change was observed in 100 per cent humidity, those in distilled water imbibing water and those in 2 per cent potassium sulfate having less stability than those in 100 per cent humidity. It is suggested that syneresis and inhibition may not be the factors primarily responsible for distortion of hydrocolloid impressions. Further evaluation is essential if these materials are to continue to increase in value in clinical dentistry.

Charbeneau and Peyton (p. 479) evaluated lubricants and cleaners for dental handpieces. With increasing speeds of operation, not only does the wear on parts become important, but the possibility of production of uncomfortable heats for patient and operator must be considered. Various lubricants were tested for velocity and lubricating ability, the latter determined by rise in bearing temperature. The effects of cleaners and belt tension were also investigated. A solvent is suggested for removing undesirable lubricants and ethyl alcohol for moisture removal. It was found that excess lubricant is undesirable, the lubricant best being applied sparingly. As still higher speeds and different types of handpieces are used, further studies in this field will be indicated.

This volume of the JOURNAL, as that of last year, is being sent to every junior dental student in the United States and, this year, Canada. This important educational project has been made possible through a grant to the JOURNAL by the Colgate-Palmolive Company. This grant, and those of others as Supporting Associates (p. 492), is an example of cooperation of industry in advancing research and education.

H. B. G. R.

ANNOUNCEMENT

The next annual meeting of the International Association for Dental Research will convene at the Hotel Statler, Detroit, Mich., on Thursday, Friday, and Saturday, March 20, 21, and 22, 1958. The Dental Materials Group of the Association will meet on Friday, Saturday, and Sunday, March 21 to 23. The American Association of Dental Schools will meet at the same place beginning on Sunday, March 23.

*Notice to Prospective Program Participants.** Abstracts of items for the program must be limited to 250 words *including* title, authors' names, etc. *Four* copies are required. Abstracts must be received by the Secretary-Treasurer *before* Nov. 4, 1957. Publication is planned.

Items submitted which do not conform to the above specifications will be returned to the author(s) without being considered for the program. If time allows they may be re-submitted.

E. H. Hatton Award Competition entries should be clearly marked as such. All co-authors must be novices.

Nominations to membership in this Association must be in the hands of the Secretary-Treasurer by February 15, 1958.

Please note change in the *official address* of the Association. All mail except that directed to specific officers and committees should be addressed as follows:

DAN Y. BURRILL, Sec.-Treas.
311 E. Chicago Avenue,
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*Members, and nonmembers sponsored by members, are invited to submit items for possible presentation on the program of the meeting.

Preprinted abstracts of papers presented at the March, 1957, meeting have been sent to those who registered. A few could not be sent because addresses on the registration cards were incomplete. If you registered and paid the registration fee but have not received the abstracts please communicate with Dan Y. Burrill, Secretary-Treasurer, 311 E. Chicago Avenue, Chicago 11, Ill.

COMPARATIVE MICROFLORA OF DEVELOPING DENTAL PLAQUES IN CARIES-IMMUNE AND SUSCEPTIBLE INDIVIDUALS

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WILLIAMS[1] and Black[2] were among the first investigators to develop generally acceptable theories concerning the composition and activity of the masses of microorganisms called dentobacterial plaques on human teeth. They considered that plaques were prerequisite for caries initiation, and that differences between cariogenic and noncariogenic plaques were due to differences in the potentialities of the bacterial populations. Modern concepts generally include these ideas.

Hemmens, Blayney, and their associates in a series of papers[3-7] presented evidence of differences between cariogenic and noncariogenic plaques. Observations on pre-carious plaques indicated a striking increase in the occurrence of lactobacilli prior to the appearance of clinically detectable carious lesions. Stephan has shown a correlation between the capacity of plaques to produce a low pH in vivo and the condition of caries activity of the individual.[8] Stralfors correlated the in vivo pH minima of plaques following a glucose rinse with the number of salivary lactobacilli.[9] From these and other studies, lactobacilli appear to be more closely associated with cariogenic potential of plaques than other members of the oral flora.

The investigations reported here are an extension of previous findings in which the most outstanding difference in the salivary flora of caries-immune and susceptible human beings was in the number of lactobacilli.[10, 11] Salivary lactobacilli were found only in very low number in caries-immune and, in significantly greater number, susceptible individuals. This was associated with an "immune mechanism" demonstrable in the saliva of caries-immune persons. In the present work, an examination of the microflora of developing plaques in caries-immune and susceptible individuals was made. Assuming that plaques from the immune persons represent noncariogenic types in contrast to those of susceptible persons, differences of the same type found in saliva, if present, may be related to the same mechanism. Considering the more direct relationship of plaque microflora to caries such evidence would point to this mechanism as the basis of the caries-immune state.

This investigation was supported by grants to the Ohio State University Research Foundation and Development Fund by the Procter and Gamble Company, Cincinnati, Ohio.
Received for publication March 30, 1956.

MATERIALS AND METHODS

The young adults used as subjects were classified on the basis of past caries activity disclosed by clinical and radiographic examinations. The caries-immune subjects had no history of caries (restorations or lesions), while the caries-susceptible subjects had clinical evidence of caries. In addition, individuals with enamel mottling (possible fluorosis) and those whose diets by choice included little or no refined sugar were exempted from the caries-immune classification.

Dental plaque material was removed by dental scaler from molar and premolar interproximal areas after gentle cleansing and forceful rinsing of the areas with a stream of water. The material was then shaken loose from the scaler into a tube containing sterile saline. The amount of material collected from the subjects was balanced visually. Dispersion of plaque material into the suspending medium was done by ultrasonic agitation in the focal point of a Brush ultrasonic generator using a hemispherical transducer. The plaque material and suspending medium were contained in a celluloid (Lustroid) tube, so positioned that the focus of the hemispherical transducer occurred in the vertical center of the column of liquid (vol. = 15 ml.). The frequency was 600 kilocycles, and energies used varied under control from 125 ma., 82 watts, to 225 ma., 263 watts. Various times of treatment were tried from 1 to 20 min., temperature was constant at 25° C.

A number of common bacteriologic media were used for qualitative studies of plaque microflora. The quantitative studies were limited to a few species, for which these were used: Hadley's tomato agar,[12] 5 per cent human blood agar, and TGE agar (Difco) plus bromeresol purple indicator, and 5 per cent CaH (PO_4) (adopted from the medium of Davies, Slack, and Tilden[13]).

RESULTS AND DISCUSSION

Standardization of the technic of ultrasonic dispersion involved first the use of pure cultures of lactobacilli to establish safe limits of treatment for maximum recovery of these organisms. Different suspending media for dispersion of plaque material were tried; distilled water, saline, saline plus 0.05 per cent sorbitan monooleate (Tween 80), and filtered sterile saliva. At least 4 plaque samples were examined in a combination of each of these suspending media with energy inputs of 125, 175, and 225 ma. Samples were withdrawn from each for plating at 2-minute intervals during 20-minute treatment. The combination of the above variables which consistently gave the largest numbers of the predominant organisms in plaque material was suspension in saline plus 0.05 per cent Tween 80, 125 ma., 82 watts input, for 4 min. at 25° C. Plaque material from several subjects was divided, one-half for ultrasonic dispersion the other for grinding with a glass mortar and pestle. In every case, greater numbers of organisms were recovered from the samples which were dispersed by ultrasound than from those dispersed mechanically.

With use of a number of common bacteriologic media for cultivation of dispersed plaque material, about 25 different types of microorganisms were readily differentiated. This diversity of plaque microflora has been reported by others and, in fact, the same species were isolated.[3, 4, 6]

Streptococci, micrococci, and lactobacilli were selected for enumeration in a quantitative study of dispersed plaque material, by reason of numbers and their possible importance in the acidogenic theory of caries. Realizing the errors inherent in visual estimation of amounts of plaque material collected, it was hoped that examination of a number of individuals might disclose any outstanding differences in plaque flora. Enumeration of these species in dispersed plaque material obtained from 26 caries-immune and 26 susceptible subjects gave the results shown in Table I. These data have been analyzed by the "t" test and found to be not significantly different at the 5 per cent level of confidence.

TABLE I

COMPARISON OF NUMBERS OF STREPTOCOCCI AND MICROCOCCI IN DENTAL PLAQUE MATERIAL FROM 26 CARIES-IMMUNE AND 26 SUSCEPTIBLE SUBJECTS

	MEANS AND STANDARD ERRORS*	
	IMMUNE	SUSCEPTIBLE
Alpha streptococci	128,000 ± 53,000	219,000 ± 95,000
Gamma streptococci	135,000 ± 57,000	145,000 ± 47,000
Acidogenic streptococci†	11,000 ± 4,000	18,000 ± 5,000
Micrococci	18,000 ± 8,000	53,000 ± 27,000

*These figures are rounded to the nearest 1,000.
†Observed as strongly acidogenic on TGE agar.

The occurrence of lactobacilli is indicated in Table II. It is obvious from these data that the presence of lactobacilli in plaque material from one area of the mouth cannot be correlated with immunity or susceptibility, however, they were found more frequently in subjects with salivary lactobacillus counts above 1,000 per millimeter. Twenty of the immune subjects had consistent low salivary counts which might indicate that whatever reduced the numbers of salivary lactobacilli in these may have also reduced the number of lactobacilli in plaque material. There was no correlation between the number of lactobacilli found in dispersed plaque suspensions and the salivary counts of the individuals from whom the material was taken, nor was there any correlation in colonial morphology between salivary lactobacilli and those found in plaque material.

TABLE II
FREQUENCY OF ISOLATION OF LACTOBACILLI FROM PLAQUE MATERIAL

SALIVARY LACTOBACILLUS COUNT	CARIES IMMUNE		CARIES SUSCEPTIBLE	
	NO. OF SUBJECTS	NO. OF ISOLATIONS	NO. OF SUBJECTS	NO. OF ISOLATIONS
<1,000	20	0	19	5
>1,000	6	5	21	9

A study of changes in some of the bacterial flora during plaque development was made by examining plaque material which had been allowed to accumulate in vivo for known periods of time. Five caries-immune subjects were

chosen, 2 of whom had very low or zero salivary lactobacillus counts, the other 3 of whom had counts of 1,000 to 10,000 per milliliter. Five caries-susceptible subjects were also examined, one of whom had a zero salivary lactobacillus count, the other 4 having counts above 10,000 per milliliter. The interproximal areas, second premolar-first molar, first molar-second molar on both right and left sides of the maxilla, were cleaned with a scaler and pumice cup. At certain intervals thereafter, plaque material was removed from these areas which had been undisturbed for periods of 24, 48, and 96 hours, and 1 week. Examination was made of the numbers of alpha, gamma, and strongly acidogenic streptococci,

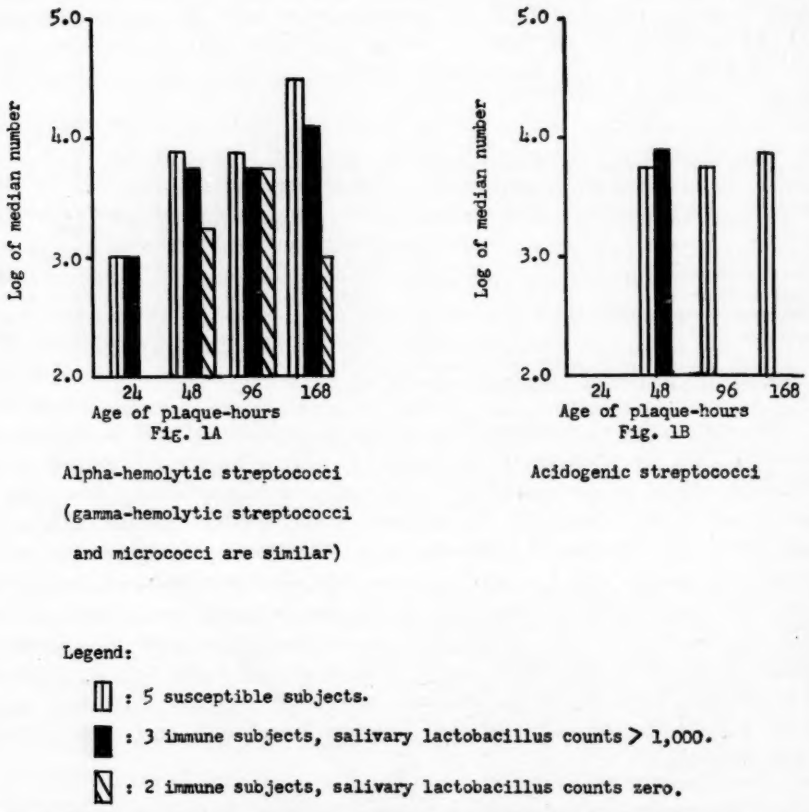

Fig. 1.—Comparative numbers of streptococci and micrococci in developing plaque material from caries-immune and susceptible individuals.

micrococci, and lactobacilli. Comparison was made of the numbers of these organisms in plaque material from caries-immune and susceptible persons, change of numbers with time, and the difference in numbers found in plaque material taken from opposite sides of the mouth.

In this study, as in Table I, the numbers of the organisms enumerated were lower in immune plaque material, but not significantly so. Again there was

no association between number or colony type of salivary lactobacilli and the corresponding data obtained from plaque material.

When the occurrence of the various organisms enumerated was plotted as the logarithm of the number found against the age of the plaque material, some differences between the immune and susceptible groups were found. Division of the immune group into those with high and those with zero salivary lactobacillus counts disclosed differences between these 2 also with respect to streptococci and micrococci. The differences among these groups in changes in numbers of streptococci and micrococci are illustrated in Fig. 1. These graphs show the change in the median number found in all plaques of each group with age of plaque material. Variation was found within each group, but the median number may be considered representative.

The changes shown in Fig. 1*A* are typical for alpha hemolytic and gamma hemolytic streptococci, and micrococci. It may be seen that the rates of increase in numbers are nearly the same for the susceptible and the high salivary count immune individuals, but the zero salivary count immune subjects exhibit an entirely different rate of change. It is interesting that these differing rates of change were found with all 3 of these groups of organisms.

Fig. 1*B* presents a different situation, for strongly acidogenic streptococci were found frequently in plaque material of caries-susceptible persons, rarely in immune individuals with high salivary lactobacillus counts, and not at all in the immune persons with zero salivary counts. The absence of both strongly acidogenic streptococci and lactobacilli in plaques of this last caries-immune group would seem to indicate a low acidogenic potential compared to the high-count caries-immune and the susceptible groups. The resemblance of the data obtained from the high salivary count caries-immune to that from the susceptible group is striking. It would appear that the conditions permitting the presence of salivary lactobacilli in these subjects are associated with presence of plaque lactobacilli and increased rate of change in numbers of streptococci and micrococci in developing plaques. These data indicate some sort of inhibition or repression of growth of streptococci and micrococci during the early stages of development of plaque material in caries-immune individuals having no salivary lactobacilli. The numbers of these organisms found in average plaques (see Table I) are similar to those found in plaque material at a known age of 96 hours to 1 week.

As noted in Table III lactobacilli were isolated from plaque material of all 5 susceptible subjects at one time or another during the period of study but less frequently from plaques of only 2 of the 5 immune subjects. These 2 latter individuals were the less typical caries-immune, having salivary lactobacillus counts of 1,000 or more and, here again, as in Fig. 1, they are more like the susceptible than the usual immune persons. The presence of large numbers of salivary lactobacilli in caries-immune individuals thus seems associated with differences in plaque flora. It seems obvious in both groups that plaque lactobacilli occur usually in individuals with high salivary counts.

TABLE III
FREQUENCY OF ISOLATION OF LACTOBACILLI FROM PLAQUE MATERIAL OF KNOWN AGES

GROUP	SALIVARY LACTOBACILLUS COUNT	NO. OF SUBJECTS*	NO. OF SUBJECTS HAVING LACTOBACILLI IN PLAQUE MATERIAL OF DIFFERENT AGES (HOURS)			
			24	48	96	168
Immune	Zero	2	0	0	0	0
Immune	>1,000	3	2	1	2	1
Susceptible	Zero	1	0	0	0	1
Susceptible	>1,000	4	2	4	4	4

*Two areas of plaque material examined in each subject.

The results of this work, like those of Blayney, Hemmens, and associates, seem to associate lactobacilli more positively with caries activity than the other types of organisms examined. The instances where lactobacilli were found in plaques but not saliva, and the converse, indicate that, in many individuals, the flora of plaques and saliva may not mirror each other. The difference in presence of lactobacilli in these sources may be one explanation why caries activity may occasionally be observed in the apparent absence of salivary lactobacilli, although this statement should not be regarded as an attempt to associate causally lactobacilli and caries.

It is interesting to note that in caries-immune persons with the low salivary lactobacillus counts usually associated with this group, the conditions producing this salivary picture also seem to be operative in the plaques of these individuals. The difference between the immune and susceptible groups seems to be also reflected in the rates of increasing numbers of several microorganisms in developing plaques. It appears that the mechanisms responsible for caries immunity exert a number of effects on the plaque flora, such that complete and partial inhibition of some species may occur.

SUMMARY

1. A method is described for dispersing dentobacterial plaque material in liquid media by means of ultrasonic agitation. The technic is simple and rapid, and gives reproducible results.

2. Comparative examination of several types of bacteria in dispersed plaque material from caries-immune and susceptible human beings showed the most striking difference to be in the low occurrence of lactobacilli in immune subjects with low salivary lactobacillus counts. The typical immune individuals have few or no lactobacilli in saliva or plaques. Plaques from those few immune subjects with high salivary lactobacillus counts resembled plaques from susceptibles.

3. Comparison of developing plaques in caries-immune and susceptible groups showed differences in rate of increase of several types of organisms, including lactobacilli. The data indicate that in immune persons with low or negative salivary lactobacillus counts, the mechanisms responsible for this condition may also affect organisms in dental plaques.

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ADDENDUM

Since the submission of this paper, it has been found that of the 6 immune subjects with high salivary lactobacillus counts (see Table II), 3 have developed clinical dental caries, 2 are now unavailable for examination, and one is currently still caries-free, but now has a low salivary count.

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LIPOLYTIC AND ESTEROLYTIC ACTIVITY OF SALIVA AND SALIVARY ORGANISMS

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THE presence of lipases in human whole saliva has been reported previously.[1-3] Salivary lipolytic activity was determined by changes in the surface tension of a saturated solution of tributyrin[4] or the hydrolysis of β-naphthyl-laurate.[5]

Although enzymes designated as lipases more readily hydrolyze the glycerides of long-chain fatty acids while esterases preferentially attack short-chain acids, both groups are capable of splitting esters of intermediate-sized chains. This overlapping has led to difficulties in terminology. Thus, esterases capable of hydrolyzing intermediate length fatty acid chains have been frequently and incorrectly labeled as lipases.

The specific aim of this project was to compare the ability of saliva and selected oral microorganisms to hydrolyze esters of short- and long-chain fatty acids. Since parotid saliva was found to contribute only a small fraction of the activity noted in whole saliva,[5] it was thought that the esterases and lipases in saliva might be predominantly bacterial in origin.

In the present study 4 esters of β-naphthol—acetate, laurate, myristate, and stearate—were employed as substrates to aid in the differentiation of the esterolytic and lipolytic properties of saliva and salivary organisms.

MATERIALS AND METHODS

Bacterial Cultures.—Microorganisms were grown in brain-heart infusion broth (Difco) at 37° C. Ten milliliter tubes, seeded with a standard inoculum, were incubated for 24 to 48 hours. The cultures were then centrifuged for 30 minutes at 3,000 r.p.m. The cell sediment was washed once with distilled water and once with bicarbonate buffer. The cell sediment and broth supernatant were tested for enzyme activity.

Substrate Solutions.—The esterolytic and lipolytic activities of saliva and salivary organisms were determined by the method of Ravin and Seligman.[6] β-naphthyl-acetate was prepared by dissolving 10.0 mg. of the substrate in 5.0 ml. of acetone. The solution was diluted to a final volume of 100 ml. with distilled water. One hundred milliliters of veronal buffer were added giving a final volume of 200 ml. of buffered substrate. β-naphthyl-laurate, β-naphthyl-myristate, and β-naphthyl-stearate solutions were prepared by

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adding 10.0 ml. of an acetone solution of each of these compounds (containing 2.0 mg. per ml.) to a mixture composed of 50.0 ml. of distilled water and 40.0 ml. of veronal buffer, pH 7.4 (0.1 M).

Estimation of Enzyme Activity.—Five milliliters of the various buffered substrates were added to the sediments and tubes containing 2.0 ml. of supernatant fluid. After 4 hours' incubation at 37° C., 1 ml. of a 2.0 mg. per milliliter solution of tetrazotized diorthoanisidine was added to each tube and 3 minutes allowed for the formation of the colored azo dye complex. One milliliter of a 40 per cent trichloroacetic acid solution was used to stop the reaction, precipitate the proteins present, and stabilize the color. Control tubes consisted of incubated buffered substrate to which the test material was added immediately before the color development. The color intensity of the control was subtracted from the experimental tube and the density of the experimental tube was estimated qualitatively. Arbitrary values were assigned to the tubes: zero indicated no demonstrable activity; ½ to 1½ showed weak activity; 2 to 4 showed moderate activity; 4½ to 7½ showed marked activity; and 8 indicated intense activity.

RESULTS

Table I shows results obtained with isolates of *Micrococcus pyogenes* var. *aureus* and *Micrococcus pyogenes* var. *albus*.

TABLE I

MICROCOCCI

ORGANISM		SUBSTRATE			
		β-N-ACETATE	β-N-LAURATE	β-N-MYRISTATE	β-N-STEARATE
M. pyogenes var. *aureus* isolate "A"	Sed	8+	5	2½	1
	Sup	8+	5	2	3
M. pyogenes var. *aureus* isolate "B"	Sed	8	7	3½	1½
	Sup	8	5	4½	6
M. pyogenes var. *albus*	Sed	7+	½	0	0
	Sup	2½	0	0	0

Micrococcus aureus isolates, "A" and "B," demonstrated a marked ability to attack β-naphthyl-acetate and β-naphthyl-laurate. The supernatant of *Micrococcus aureus* "B" attacked all 4 substrates vigorously; the hydrolysis of

TABLE II

LACTOBACILLI

ORGANISM		SUBSTRATE			
		β-N-ACETATE	β-N-LAURATE	β-N-MYRISTATE	β-N-STEARATE
L. casei	Sed	8	8	3	0
	Sup	1	0	0	0
L. brevis	Sed	2½	½	0	0
	Sup	1½	0	0	0
L. *plantarum*	Sed	6	1½	½	0
	Sup	2	0	0	0

stearate was even more pronounced than that of myristate. Such a reaction indicated the presence of a true lipase. In contrast, *Micrococcus albus* was able to attack only the acetate.

Forty-eight hour cultures of *Lactobacillus casei, L. brevis,* and *L. plantarum* were examined for estrolytic and lipolytic activity (Table II).

Only *L. casei* cells showed any propensity to break down the higher chain compounds. The activity was moderate with myristate and absent when stearate was employed as substrate.

Two isolates of *Streptococcus mitis* (A_9 and 19_c); *Str. salivarius,* a Lancefield group "L," and one Lancefield group "A" streptococcus were tested (Table III). Of these 5 organisms only the 2 strains of *Str. mitis* showed more than weak activity, evident only in the sediment. Both strains exhibited a decrease when tested against high fatty acid moieties.

TABLE III
STREPTOCOCCI

ORGANISM		SUBSTRATE			
		β-N-ACETATE	β-N-LAURATE	β-N-MYRISTATE	β-N-STEARATE
Str. mitis 19_c	Sed	8	2	1½	0
	Sup	2	½	0	0
Str. mitis A_9	Sed	8	8	6	2
	Sup	2	0	0	0
Lancefield group A streptococcus	Sed	1½	0	0	0
	Sup	0	0	0	0
Lancefield group L streptococcus	Sed	2	0	0	0
	Sup	1½	0	0	0
Str. salivarius	Sed	1	0	0	0
	Sup	0	0	0	0

Of the miscellaneous organisms listed in Table IV, the reaction of *Aerobacter aerogenes* appeared of interest. The sedimented cells showed intense activity against only the acetate substrate. A reaction of this type suggested the presence of an enzyme capable of splitting only acetic acid esters but no ability to hydrolyze esters with longer carbon chains. Two types of enzymes are

TABLE IV
MISCELLANEOUS ORGANISMS

ORGANISM		SUBSTRATE			
		β-N-ACETATE	β-N-LAURATE	β-N-MYRISTATE	β-N-STEARATE
A. aerogenes "M"	Sed	6½	4	½	0
	Sup	2	0	0	0
A. aerogenes	Sed	8	0	0	0
	Sup	1½	0	0	0
N. catarrhalis	Sed	1	0	0	0
	Sup	1½	0	0	0
D. pneumoniae II	Sed	2	1	0	0
	Sup	4½	3	1	0
C. albicans	Sed	6½	1	0	0
	Sup	1½	0	0	0

known to fit this assumption, acetylesterases and cholinesterases. When cell sediments of *Aerobacter aerogenes* and *Micrococcus albus,* which showed similar behavior, were incubated with β-carbonaphthoxycholine iodide, hydrolysis occurred indicating the ability of these organisms to elaborate a pseudocholinesterase. A negative reaction was obtained with *Candida albicans* although this organism followed the pattern of strong acetate hydrolysis and inability to attack the longer carbon chains.

Table V indicates the ability of saliva to attack the 4 β-naphthol substrates. Both salivary sediment and supernatant fluid showed an intense to marked reaction when tested against β-naphthyl-acetate. A marked to moderate activity was evident against the laurate substrate. With the longer chain compounds, a decrease in hydrolytic power was apparent. The attack on myristate was moderate and the reaction with the stearate substrate weak or absent.

TABLE V

SALIVA

SUBJECT		SUBSTRATE			
		β-N-ACETATE	β-N-LAURATE	β-N-MYRISTATE	β-N-STEARATE
No. 1	Sed	8	5½	3½	1
	Sup	8	5	3	2½
No. 2	Sed	8	2½	½	½
	Sup	8	4½	2	1½
No. 3	Sed	8	3½	2½	1
	Sup	8	3	1	½
No. 4	Sed	8	[illegible]	½	0
	Sup	8	7	3	0
No. 5	Sed	8	3	2	0
	Sup	8	7½	3	0

DISCUSSION

Overlapping in substrate attack occurs among esterases. In such instances marked or intense activity against β-naphthyl-acetate is evident. Some hydrolysis of the higher chain fatty acids should then be expected but with a decreasing intensity in the following order: acetate, laurate, myristate, and stearate. This typical pattern occurs with many of the organisms tested, *Lactobacillus casei, L. plantarum, Streptococcus mitis* 19_c, *Str. mitis* A_9, *Aerobacter aerogenes M,* and *Diplococcus pneumoniae, type II.* However, with certain other bacteria, moderate to marked activity occurred with the higher fatty acid carbon compounds. With both isolates of *Micrococcus pyogenes* var. *aureus* a greater activity was noted with stearate than with the myristate. Such behavior would indicate the presence of a true lipase. The ability of these organisms to hydrolyze fats was confirmed with the copper sulfate butter plate method.[7] The fact that saliva contains only minute amounts of lipase at best is shown by the feeble hydrolysis of both myristate and stearate. This is not surprising since the lipase producing gram-positive micrococci occur in the oral cavity in relatively small numbers. The marked salivary hydrolysis of

β-naphthyl-acetate and β-naphthyl-laurate can be explained by the presence of lactobacilli such as *L. casei* and most probably by the α-hemolytic streptococci which usually are present in very high numbers.

SUMMARY

1. Saliva and 16 oral isolates were tested for lipolytic and esterolytic activity using β-naphthyl-acetate, β-naphthyl-laurate, β-naphthyl-myristate, and β-naphthyl-stearate.

2. True lipase activity was shown only by isolates of *Micrococcus pyogenes* var. *aureus*.

3. Saliva, 3 species of lactobacilli and certain of the gram-positive cocci exhibited an overlapping of enzymatic activity which manifested itself in a strong attack against β-naphthyl-acetate and a decreasing hydrolysis of β-naphthyl-laurate, β-naphthyl-myristate, and β-naphthyl-stearate.

4. *Micrococcus pyogenes* var. *albus* and an isolate of *Aerobacter aerogenes* hydrolyzed only the acetate substrate. When tested against β-carbonaphthoxycholine iodide these organisms indicated the production of a pseudocholinesterase.

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THIAMINE CL-HCL EFFECT UPON ACID PRODUCTION AND UPON DEXTRINIZING TIME OF SALIVARY AMYLASE WITH CORN STARCH SUBSTRATE

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A RELATIONSHIP between the speed with which salivary amylase breaks corn starch to a product not giving blue or purple color with iodine and dental decay experience has been pointed out by several investigators.[1-4]

Wohlgemuth's[5] experiments indicated that both fluoride and certain amino acids change the speed with which pancreatic amylase breaks starch to a form failing to give blue or purple color with iodine. Sherman and his colleagues[6] demonstrated clearly that certain amino acids, upon being added to amylase, speeded its action on starch, while other amino acids failed to speed the amyloclastic phase of starch breakdown.

Enzymes, in addition to their amino acid chain, frequently utilize a vitamin as their prosthetic group. It seemed worth-while, therefore, to determine whether, using corn starch as the substrate, some vitamin would alter the speed of salivary amylase activity.

In the study being reported here, we have further confirmed the faster dextrinization of corn starch by saliva from individuals with high proneness to dental decay. In addition, the results of this investigation indicate that the addition of vitamin B_1 (thiamine Cl-HCl) to saliva fairly consistently speeds the dextrinization of corn starch by salivary amylase. The degree to which this time was speeded differed for the various groups, classified according to their dental decay activity.

The method of estimating caries extent and activity[7] in these subjects was by a per cent activity classification based on the previous 6-month period, that is, the number of surfaces with newly formed lesions was used as the numerator, and the number of surfaces that were available for decay was used as the denominator. The result, expressed as a per cent, was used as the current activity index.

The subjects for this study were children whose chronological ages were primarily 4 to 12 years (extreme range in age was 3 yrs. 7 mos. to 15 yrs. 3 mos.). The children were from Boston and its suburbs and were brought to the Forsyth Dental Infirmary for treatment. The group included children with extensive dental decay and children free from dental decay.

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METHODS AND RESULTS

A. *Dextrinizing test:* One milliliter of 1 per cent corn starch (nonsoluble) was added to 1 ml. of freshly given "resting" saliva, i.e., saliva obtained without artificial stimulation. The test tube containing the mixture was shaken (manually) and immediately immersed in a constant temperature bath, set at 37° C. At given time intervals, 1 drop of the mixture was withdrawn and placed on strips of filter paper previously treated with 1 per cent potassium iodide. A drop of 3 per cent hydrogen peroxide was placed over the starch-saliva spot, and the color was noted under a white fluorescent lamp immediately after the absorption of the liquids into the paper. The absence of blue or lavender coloration was taken as an indication of the completion of the amyloclastic phase of starch digestion.

This method brings dextrinizing times closer together than had the former spot plate[2] and test tube methods[3] but does not obliterate inter-group differences. (At one time in this laboratory, salivary amylase action was tested by keeping the time constant and letting the amount of starch digested, as read by means of a Photelometer, be the variable. With that method we obtained definite inter-individual differences as had Pratt and Eisenbrandt.[8] However, careful analysis of the results of that test for 250 children indicated that when we substituted this method we lost the association with dental decay activity.)

From analysis of these data it is apparent that a significant association exists between dextrinizing time of salivary amylase with corn starch and dental decay activity (Table I).

TABLE I

MEAN VALUES FOR DEXTRINIZING TIME WITH B_1 ADDED

DENTAL DECAY ACTIVITY (%)	NO. OF CHILDREN	MEAN DEXTRINIZING TIME WITHOUT B_1 (MIN.)	MEAN DEXTRINIZING TIME WITH B_1 ADDED (MIN.)
0	27	36.41	21.33
0.1-9.0	94	29.70	18.75
9.1+	14	20.86	15.86

B. *The dextrinizing test* was carried out for 135 individuals, tested for dextrinizing time without added B_1, with the addition of 0.5 ml. of 1 per cent thiamine Cl-HCl (freshly prepared) to the 1 ml. of saliva just prior to the addition of the 1 ml. of 1 per cent corn starch. The same procedures as outlined above were used for determining dextrinizing time, and the results are summarized in Tables I and II.

In Table II, the results show the speeding of dextrinizing time (expressed as a per cent) upon the addition of vitamin B_1 to a saliva–corn starch mixture.

The addition of vitamin B_1 speeded the dextrinizing time for the saliva of 131 of the 135 cases tested (Fig. 1). Of the 4 cases where the dextrinizing time with and without B_1 added were the same, 3 occurred among the children

with a 9.1+ per cent dental decay activity, while one was in the group 0.1 to 9.0 per cent. (The mean speeding for the 135 cases was 35.16 per cent [±13.92]; the range was from 0 to 67 per cent.)

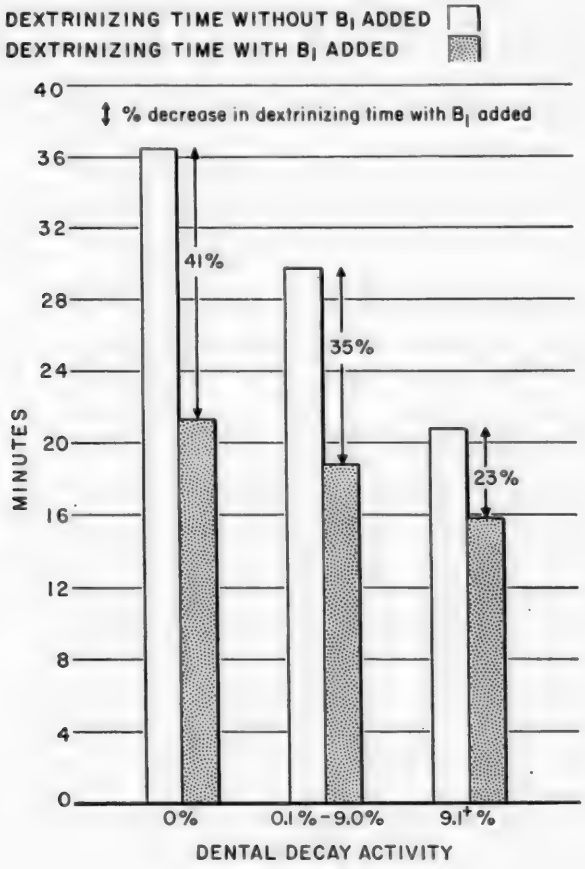

Fig. 1.

TABLE II

PERCENTAGE SPEEDING OF DEXTRINIZING TIME UPON THE ADDITION OF THIAMINE CL-HCL

DENTAL DECAY ACTIVITY (%)	NO. OF CHILDREN	SHORTENING OF REACTION TIME (%)	SIGMA
0	27	41.41	±13.03
0.1-9.0	94	35.12	±12.82
9.1+	14	23.36	±14.97

"t" values and confidence levels for significance of the above results are shown below:

Activity (%)	"t"	Confidence Level (%)
0 vs 9.1+	3.90	>99
0 vs 0.1-9.0	2.22	<98
0.1-9.0 vs 9.1+	3.10	>99

C. *Titratable acidity* was measured for the saliva–corn starch mixture of each subject after a 2-hour incubation period in a constant temperature bath at 37° C. The amounts used were as above—1 ml. saliva and 1 ml. of 1 per cent corn starch. The figure for titratable acidity represents the amount of 0.001N NaOH required to bring the saliva–corn starch mixture to a pH of 8.5 on the Beckman pH meter using external electrodes. The results are shown in Table III.

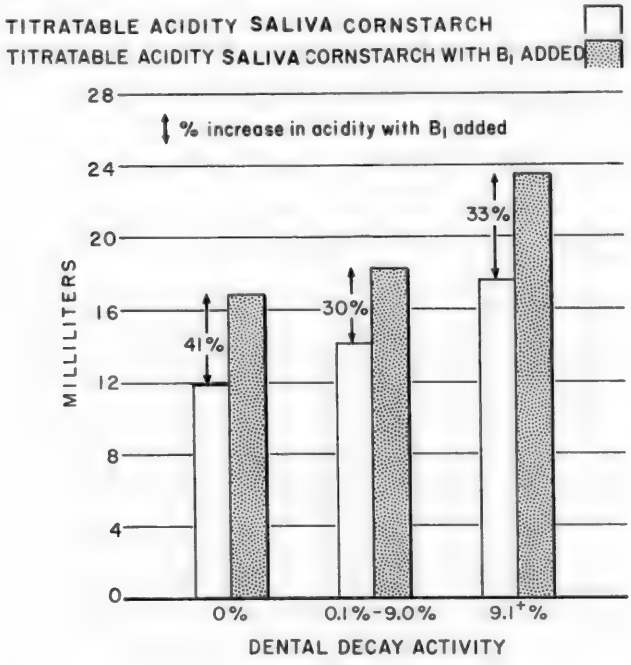

Fig. 2.

TABLE III

TITRATABLE ACIDITY FROM SALIVA–CORN STARCH FOR THE GROUPS STUDIED

DENTAL DECAY ACTIVITY (%)	NO. OF CHILDREN	MEAN ACIDITY (ML.)	SIGMA
0	40	11.99	±6.52
0.1-9.0	147	14.05	±7.08
9.1+	24	17.62	±6.97

"t" values and confidence levels for significance of the above results are shown below

Activity (%)	"t"	Confidence Level (%)
0 vs 9.1+	3.24	>99
0 vs 0.1-9.0	1.65	=90
0.1-9.0 vs 9.1+	2.29	>98

When the extreme groups in dental decay activity are contrasted, it appears that titratable acidity from saliva–corn starch, after a 2-hour incubation period, distinguishes the groups at a 99 per cent confidence level.

Titratable acidity of individual salivas was also determined following incubation with corn starch to which 0.5 ml. of 1 per cent vitamin B_1 had been added.

As shown in Tables III and IV and in Fig. 2, the titratable acidity was increased by the addition of B_1 before incubation. However, the titratable acidity with B_1 added was not as much as the sum of titratable acidity of the corn starch–saliva mixture without B_1 plus the titratable acidity of the amount of B_1 added.

TABLE IV
TITRATABLE ACIDITY FROM SALIVA, CORN STARCH WITH B_1 ADDED

DENTAL DECAY ACTIVITY (%)	NO. OF CHILDREN	MEAN ACIDITY (ML.)	SIGMA
0	31	16.92	±6.11
0.1-9.0	90	18.20	±5.44
9.1+	11	23.48	±7.15

DISCUSSION

One's attention is naturally directed to the chloride of thiamine chloride hydrochloride since it has been clearly established that chloride is an activator for salivary amylase.[9, 10] Moreover, the salivary chloride level has been found to be associated with dental decay proneness.[11] Lest the effect of thiamine Cl-HCl was only that of chloride, equimolar chloride as sodium chloride was added to the saliva of a test series. A speeding of dextrinizing time comparable to that of thiamine Cl-HCl was not found with sodium chloride.

However, the possibility that this in vitro effect may be strongly influenced by the lowered pH naturally suggests itself. Investigation of this point is under way. Preliminary tests indicate that hydrochloric acid added to saliva and corn starch to a pH equivalent to that of saliva, corn starch, and thiamine chloride hydrochloride, also speeds dextrinizing time. It is interesting to note that the pH of the saliva–corn starch–thiamine chloride hydrochloride mixture is well below the commonly quoted optimum pH for salivary amylase of 6.9. We have found that the pH of parotid saliva may be as low as 5.5 (average for 50 children, 5.95).[12] The parotid saliva of these children dextrinized starch very much faster than did their whole saliva.

SUMMARY

a. This study gives further evidence that the dextrinizing time, resulting from the action of salivary amylase on corn starch, and dental decay proneness are associated.

b. The addition of vitamin B_1 (thiamine Cl-HCl) to the saliva–corn starch mixture speeds the dextrinizing time. The speeding (expressed as a per cent) is significantly greater for the caries-resistant group than for the group which has experienced recent rapid dental decay activity.

c. The amount of titratable acidity from saliva and corn starch differs for the dental caries-free and caries-active groups.

d. When vitamin B_1 is part of the saliva–corn starch reaction mixture during incubation, the titratable acidities are higher in all groups than for saliva–corn starch alone, though not as high as the acidity figure for saliva–corn starch, plus the acidity figure for thiamine Cl-HCl.

e. The question is raised whether pH 6.9 is in fact the optimum pH for the amyloclastic phase of salivary amylase in such a medium as saliva–corn starch.

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COMPARATIVE STUDIES ON THE INFLUENCE OF ANTIBIOTIC SUBSTANCES IN DENTAL CARIES IN THE WHITE RAT AND THE COTTON RAT

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THE effect of antibiotics on experimental caries may be classified according to the degree of inhibition that is produced. Comparative tests of different types of antibiotics under comparable circumstances may serve as a convenient procedure for studies on the etiology of tooth decay. Generally speaking, those antibiotics which are most effective against gram-positive microorganisms have had the most influence upon the initiation and progression of carious lesions. Previous investigators have shown that the incorporation of penicillin into a cariogenic diet was responsible for major reduction in caries in white rats and hamsters.[1, 2] Aureomycin, streptomycin, and Chloromycetin have also been shown to be effective, but to a lesser degree.[2] A more rigorous set of conditions for testing the efficacy of inhibitory agents in the prevention of dental caries can be obtained by removal of the salivary glands, thus greatly increasing the susceptibility of the experimental subjects to dental caries.

The studies to be reported in this paper were designed primarily to compare the relative caries-inhibiting activity of different antibiotics and other compounds with pronounced bacteriostatic properties in the intact and desalivated rats as a means to explore the etiology of caries. In addition a variety of in vitro tests were conducted with some of the antibiotic materials in order to determine to what extent there might be a correlation between in vivo and in vitro experiments.

EXPERIMENTAL PROCEDURE

The following antibiotic materials were tested in a series of ten animal experiments: animal protein factor concentrate (APF-5), Lederle Laboratories Division, American Cyanamide Co.; crystalline Aureomycin hydrochloride, Lederle Laboratories Division, American Cyanamide Co.; bacitracin, powdered, Control No. 1880-LIF, S. B. Penick & Co.; Chloromycetin, Lot No. 131340, Parke, Davis & Co.; indole, Distillation Products Inc.; Panthenol, Hoffmann-La Roche, Inc.; penicillin, used as Duracillin fortified, which is a mixture of 300,000 units of crystalline procaine penicillin-G and 100,000 units of buffered penicillin-G crystalline sodium, Eli Lilly & Co.; streptomycin sulfate, Parke,

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Davis & Co.; subtilin, Control No. 326, Western Regional Research Laboratory; crystalline Terramycin hydrochloride, Charles Pfizer & Co., Inc.; tyrothricin, U.S.P. XIV, Control No. 1090-LIF, S. B. Penick & Co.

The first experiment was conducted in 2 parts with intact white rats and with intact cotton rats. The first of the 3 groups for each species received the highly cariogenic purified Ration 100,[3] without any added antibiotic supplement. The subjects in the second and the third groups of each part of the experiment received supplements in the diet of 0.05 per cent penicillin and 0.05 per cent Aureomycin, respectively. The second experiment was conducted with white rats from which the principal salivary glands had been removed. This experiment consisted of 3 groups. The rats in the first group received Ration 100 and no antibiotic supplement while rats in the second and third groups received supplements of 0.01 and 0.025 per cent penicillin, respectively. The subjects in the 3 groups of the third experiment were sialo-adenectomized rats. The first group received the basal Ration 100 only, and the animals in the second and third groups received 0.05 per cent penicillin and 0.05 per cent Aureomycin, respectively. The fourth experiment consisted of 2 parts with 3 groups in each part. The subjects in the first part of the experiment were intact white rats, while those in the second part were sialo-adenectomized rats. The first group in each half of the experiment received the basal ration only, while the second and third groups received 1 and 2 per cent of the animal protein factor concentrate, respectively.

The fifth, sixth, and seventh experiments were conducted with sialo-adenectomized white rats. The first group in each experiment received the basal ration only. The rats in the second and third groups of the fifth experiment received 0.01 and 0.05 per cent subtilin, respectively. The rats in the second and the third groups of the sixth experiment received 0.05 per cent Aureomycin and 0.05 per cent Chloromycetin, respectively. The subjects in the second and third groups of the seventh experiment received supplements of 0.05 per cent tyrothricin and 0.05 per cent bacitracin, respectively.

The eighth experiment was conducted with both sialo-adenectomized white rats and with intact cotton rats. The first group of subjects from each species received the basal ration only. The second and third groups for each species received supplements of streptomycin and Terramycin, respectively, at levels of 0.05 per cent. The ninth experiment was conducted with intact white rats and with intact cotton rats. The first groups of each species in the experiment received the basal ration. The second, third, and fourth groups for each species received supplements of indole at levels of 0.5, 1.0, and 2.0 per cent, respectively. The tenth experiment was conducted with intact white rats and with sialo-adenectomized white rats to test the influences of Panthenol supplements on dental caries incidence. The basal ration in this case was Ration 100 from which calcium pantothenate had been omitted. The first group in each species received a supplement of 20 mg. of calcium pantothenate per kilogram of diet, an amount adequate to provide normal growth. The subjects in the second group of each species received 300 mg. of Panthenol per kilogram of the pantothenic

acid-deficient diet. The third group for both white and cotton rats received the pantothenic acid-deficient diet plus a supplement of 150 mg. of Panthenol per liter of drinking water. The cotton rats and the white rats in the fourth groups received supplements of 300 mg. of Panthenol per kilogram of diet, and also supplements of 150 Gm. of Panthenol per liter of drinking water.

The experimental subjects used in these experiments were offspring of the caries-susceptible strains of white rats and cotton rats maintained in our laboratories. The breeding colony of white rats was maintained on Ration 100 throughout its entire reproductive life; the breeding colony of the cotton rat strain was maintained on a natural diet because of the inability to breed adequately on a purified diet. Offspring of the white rats were weaned at 21 days of age and immediately placed on experiment. Littermates were distributed as evenly as possible among the groups within an experiment. The cotton rats were weaned at 14 days of age and also distributed among the experimental groups according to littermates. Wherever intact white rats were used in these experiments, the experimental period was 140 days. When sialo-adenectomized animals were to be used, the young rats were allowed to accustom themselves to the experimental circumstances for 3 to 5 days before the principal salivary glands, parotid, submaxillary, major sublingual, and extraorbital-lacrimal, were removed.

The experimental duration among these rats varied from 70 to 85 days after operation, depending upon the rate at which carious lesions were visually observed to be initiated. The cotton rats in all these experiments were maintained for a period of 100 days on experiment. Throughout the experimental period, each rodent was maintained in a single wire-bottom cage and was fed the appropriate ration and drinking water ad libitum. At the termination of the experiments, the animals were sacrificed under ether anesthesia and the skulls fixed in 95 per cent alcohol. The number of carious molars, the number of carious lesions and the average extent of carious lesions were evaluated and recorded according to the procedure reported by Shaw, Schweigert, McIntire, and Elvehjem.[4]

While these animal experiments were in progress, certain types of in vitro studies were undertaken. These were designed to test the amount of antibiotic required to achieve 80 per cent of inhibition of acid production by representative strains of lactobacilli that were isolated from the saliva from highly caries-susceptible human subjects or from swab samplings taken in the mouths of highly caries-susceptible white rats. After several dilutions of the oral samples, tomato juice agar plates[5] were prepared and incubated for 72 hours. From these plates, individual cultures were transferred to a culture medium designed for this type of experimental test by Weisberger.[6] In the early stages of this study, the isolates were characterized by acceptable bacteriologic procedures and only *L. acidophilus* strains of high acid production were maintained. All the actual assays of antibiotic activity were conducted with 2 strains; one was the most rapid acid-former isolated from human saliva, the second was the most rapid acid-former isolated from the oral cavity of a rat.

The assays were conducted in tubes containing 10 ml. of Weisberger's medium. Graded levels of a given antibiotic were introduced under sterile conditions into the assay tubes. Each level of antibiotic was tested in triplicate tubes. Inoculation was made by loop from a young culture. The assay tubes were incubated for 48 hours after which the amount of acid produced in each tube was titrated with 0.1 N. NaOH.

RESULTS

The results from the first experiment are presented in Table I. The growth of both intact male and female white rats fed the 0.05 per cent penicillin and 0.05 per cent Aureomycin in supplements was equivalent to that of the rats in the control group. The 0.05 per cent penicillin supplement in the diet caused significant reductions in the average number of carious teeth, the average number of carious lesions and the average extent of carious lesions. The 0.05 per cent supplement of Aureomycin did not cause reductions which were statistically significant, although there was a substantial trend toward caries

TABLE I
THE EFFECT OF DIETARY SUPPLEMENTS OF PENICILLIN AND AUREOMYCIN ON THE DENTAL CARIES INCIDENCE OF INTACT WHITE RATS AND COTTON RATS

Ration Supplement	No. of Rats	Total Increment in Body Wt. ♀ gm.	Total Increment in Body Wt. ♂ gm.	Number of Carious Teeth Avg.	Number of Carious Teeth SEM*	Number of Carious Teeth CR†	Number of Carious Lesions Avg.	Number of Carious Lesions SEM*	Number of Carious Lesions CR†	Extent of Carious Lesions Avg.	Extent of Carious Lesions SEM*	Extent of Carious Lesions CR†
White Rats												
None	10	181	270	4.0	0.9	2.5	4.9	1.5	2.2	9.2+	2.5+	2.9
0.05 % Penicillin	9	215	270	1.4	0.5	0.7	1.4	0.5	0.9	1.8+	0.6+	1.7
0.05% Aureomycin	10	209	268	3.2	0.7	2.1	3.3	0.8	2.0	4.6+	1.2+	2.1
Cotton Rats												
None	5	37	41	5.3	1.0	5.3	10.6	3.0	3.5	24.0+	8.1+	3.0
0.05% Penicillin	3	21	20	0.0	0.0	0.5	0.0	0.0	0.4	0.0	0.0	0.3
0.05% Aureomycin	5		37	4.6	0.8	5.7	9.2	2.2	4.2	21.4+	6.1+	3.5

* SEM--Standard Error of Mean

† CR--Critical Ratio. The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Wherever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; when from 2.0 to 2.9, moderately significant; when 3.0 or higher, highly significant.

reduction among this group of intact white rats. In the second part of the experiment where intact cotton rats were used as experimental subjects, the supplement of 0.05 per cent penicillin appeared to be mildly toxic as noted from the average rate of growth for the experimental subjects. In addition, 2 animals in this group died during the experimental period. This level of penicillin resulted in a complete prevention of tooth decay in this species. The 0.05 per cent supplement of Aureomycin did not cause any reduction in the rate of growth and caused only minor reductions in the dental caries incidence which were not judged to be statistically significant.

The results of Experiment 2 are presented in Table II. Supplements of 0.01 and 0.025 per cent penicillin did not cause any demonstrable changes in the increase in body weight of these experimental subjects during the full period of the study. However, both supplements produced striking reductions of comparable degree in the 3 criteria used for judging the dental caries incidence of the sialo-adenectomized white rats. In both cases the reductions were statistically highly significant. The results of the third experiment are presented in Table II. Again the supplements of 0.05 per cent penicillin and 0.05 per cent Aureomycin were not responsible for alterations in the rate of growth among these animals. The low increments in body weight observed among the sialo-adenectomized rats in Experiments 2 and 3 were occasioned by the shorter trial period than in Experiment 1 and by the lower rate of growth in sialo-adenectomized rats than in intact rats. The 0.05 per cent

TABLE II
THE INFLUENCE OF AUREOMYCIN AND OF VARIOUS LEVELS OF PENICILLIN IN THE DIET ON THE DENTAL CARIES INCIDENCE IN SIALO-ADENECTOMIZED WHITE RATS

Ration Supplement	No. of Rats	Total Increment in Body Wt.		Number of Carious Teeth			Number of Carious Lesions			Extent of Carious Lesions		
		♀ gm.	♂ gm.	Avg.	SEM*	CR†	Avg.	SEM*	CR†	Avg.	SEM*	CR†
None	7	84	166	11.7	0.3	4.3	26.6	1.5	7.1	104.3+	12.0+	6.4
0.01% Penicillin	8	107	156	5.6	1.4	3.7	8.4	2.1	7.1	18.1+	6.1+	6.5
0.025% Penicillin	7	67	157	6.0	1.5	0.2	8.3	2.1	0.3	18.3+	5.4+	0.2
None	8	93	121	11.5	0.5	12.4	26.0	1.4	15.6	76.3+	5.9+	11.9
0.05% Penicillin	9	89	122	2.7	0.5	4.4	2.7	0.5	6.1	4.9+	1.1+	7.9
0.05% Aureomycin	8	87	115	7.0	0.9	4.2	11.5	1.9	4.5	22.4+	3.4+	4.9

* SEM -- Standard Error of Mean
† CR -- Critical Ratio

supplement of penicillin caused a highly significant reduction in the incidence of dental caries. The percentage reduction in tooth decay among these sialo-adenectomized rats was approximately comparable to the reductions caused by the same supplement in intact rats. The reductions caused by the 0.05 per cent Aureomycin were much less striking than those caused by the penicillin supplements; however, the Aureomycin produced reductions were also statistically highly significant. The degree of reduction caused in the sialo-adenectomized rats was somewhat greater than the same supplement produced in intact rats.

The results of Experiment 4 are presented in Table III. Neither the 1 per cent nor the 2 per cent supplement of animal protein factor concentrate influenced the rate of growth nor the dental caries incidence among intact rats to any significant degree. However, at the 2 per cent level, there was a slight trend toward a lower dental caries incidence. Among the sialo-adenectomized rats in the second part of the experiment, again there was no striking influence

on the body weights by either of the supplements. However, with both 1 per cent and 2 per cent animal protein factor concentrate supplements, there was a substantial trend toward a reduction in all 3 criteria used for judging the incidence of tooth decay. These reductions reached statistical significance for the differences between the average number of carious lesions and the average extent of carious lesions for the control animals and for the rats in the 2 per cent animal protein factor concentrate group. The difference between the average extent of carious lesions in the control and 1 per cent animal protein factor groups was moderately significant. In view of the trend in both intact and sialo-adenectomized rats, it seems likely that there is a definite caries-inhibiting influence produced by the animal protein factor concentrate.

TABLE III

THE INFLUENCE OF A FERMENTATION CONCENTRATE CONTAINING AUREOMYCIN AND VITAMIN B_{12} ON THE DENTAL CARIES INCIDENCE OF INTACT AND SIALO-ADENECTOMIZED WHITE RATS

Ration Supplement	No. of Rats	Total Increment in Body Wt. ♀ gm.	Total Increment in Body Wt. ♂ gm.	Number of Carious Teeth Avg.	Number of Carious Teeth SEM*	Number of Carious Teeth CR†	Number of Carious Lesions Avg.	Number of Carious Lesions SEM*	Number of Carious Lesions CR†	Extent of Carious Lesions Avg.	Extent of Carious Lesions SEM*	Extent of Carious Lesions CR†
Intact Rats												
None	3	155	226	7.3	2.0	0.0	8.7	2.9	0.0	24.7+	7.5+	0.3
1% APF Concentrate	5	127	260	7.4	1.2	0.7	8.6	2.8	0.2	21.0+	8.3+	0.8
2% APF Concentrate	4	130	146	6.0	0.0	1.2	8.0	2.1	0.2	16.8+	4.3+	0.5
Sialo-adenectomized Rats												
None	12	130	175	11.8	0.2	0.8	26.8	0.8	1.2	110.3+	4.3+	2.1
1% APF Concentrate	11	150	208	11.5	0.3	1.1	25.5	0.7	2.3	96.3+	5.2+	3.5
2% APF Concentrate	11	160	215	11.3	0.4	0.4	23.5	1.2	0.2	79.4+	7.7+	1.8

* SEM --Standard Error of Mean

† CR -- Critical Ratio

The results of Experiment 5 are presented in Table IV. A supplement of 0.01 per cent subtilin seemed to stimulate the rate of growth of sialo-adenectomized white rats appreciably but did not cause any reduction in the dental caries incidence. The higher level of 0.05 per cent did not promote any increased increment in body weight, but did cause minor decreases in dental caries incidence which were not statistically significant.

The results of Experiment 6 are presented in Table IV. The supplement of 0.05 per cent Aureomycin gave reductions in dental caries incidence that were statistically significant at approximately the same level observed in Experiment 2. The supplement of 0.05 per cent Chloromycetin caused decreases in the dental caries incidence which were statistically highly significant but not at as high levels of significance as was observed with the Aureomycin supplement. Neither of these antibiotics caused any appreciable effect upon the average increment in body weight during the experimental period.

The results of Experiment 7 are presented in Table IV. The supplement of 0.05 per cent tyrothricin caused minor reductions in the increment in body weight among sialo-adenectomized white rats and caused no reduction whatever in the dental caries incidence. The supplement of 0.05 per cent bacitracin had no influence on the increment in body weight and caused minor reductions in the dental caries incidence. The difference for average extent of carious lesions was statistically significant at the 5 per cent level.

TABLE IV
THE INFLUENCE OF SUBTILIN, AUREOMYCIN, CHLOROMYCETIN, TYROTHRICIN AND BACITRACIN ON THE DENTAL CARIES INCIDENCE OF SIALO-ADENECTOMIZED WHITE RATS

Ration Supplement	No. of Rats	Total Increment in Body Wt.		Number of Carious Teeth			Number of Carious Lesions			Extent of Carious Lesions		
		♀ gm.	♂ gm.	Avg.	SEM*	CR†	Avg.	SEM*	CR†	Avg.	SEM*	CR†
None	13	121	166	11.9	0.1	1.6	25.3	0.7	0.0	76.8+	3.9+	0.2
0.01% Subtilin	13	142	213	11.4	0.3	2.4	25.1	1.3	1.5	78.2+	6.2+	1.0
0.05% Subtilin	14	127	145	10.2	0.7	1.5	21.4	2.2	1.4	67.0+	8.5+	1.1
None	8	85	137	11.4	0.5	3.6	26.0	1.4	11.5	84.5+	7.9+	6.5
0.05% Aureomycin	7	104	147	5.7	1.5	3.0	8.0	0.7	3.4	19.7+	6.0+	3.5
0.05% Chloromycetin	8	99	131	7.0	1.4	0.6	13.0	3.5	1.4	36.0+	11.2+	1.3
None	15	85	141	11.3	0.4	0.0	23.7	1.8	0.3	75.9+	10.2+	0.0
0.05% Tyrothricin	16	79	102	11.3	0.3	1.4	24.3	1.4	1.8	75.3+	7.0+	2.0
0.05% Bacitracin	14	82	133	10.4	0.5	1.6	19.5	1.5	2.2	52.4+	5.2+	2.6

* SEM --Standard Error of Mean
† CR -- Critical Ratio

The results of Experiment 8 are recorded in Table V. Supplements of 0.05 per cent streptomycin and 0.05 per cent Terramycin to desalivated white rats had no influence on the rate of growth. The supplement of streptomycin appeared to cause minor reductions in dental caries incidence which were not statistically significant. The supplement of Terramycin caused slight reductions in dental caries incidence which were statistically significant for the average number of carious teeth and for the average number of carious lesions, and highly significant for the average extent of carious lesions. Among the cotton rats in the second part of this experiment, it appeared that a reverse influence had been demonstrated. The supplement of 0.05 per cent streptomycin caused reductions in dental caries incidence which were highly significant, whereas the 0.05 per cent Terramycin supplement caused minor increases which were statistically insignificant. In addition, the level of Terramycin supplementation was sufficiently toxic to cause the death of 7 of the original 12 subjects and to reduce appreciably the increment in body weight of the remainder.

TABLE V

THE INFLUENCE OF STREPTOMYCIN AND TERRAMYCIN SUPPLEMENTS ON THE DENTAL CARIES INCIDENCE OF SIALO-ADENECTOMIZED WHITE RATS AND INTACT COTTON RATS

Ration Supplement	No. of Rats	Total Increment in Body Wt. ♀ gm.	Total Increment in Body Wt. ♂ gm.	Number of Carious Teeth Avg.	SEM*	CR†	Number of Carious Lesions Avg.	SEM*	CR†	Extent of Carious Lesions Avg.	SEM*	CR†
Sialo-adenectomized White Rats												
None	15	114	150	11.9	0.2	1.2	26.0	0.7	1.1	86.3+	5.5+	1.9
0.05% Streptomycin	13	110	159	11.5	0.2	2.4	24.7	1.0	2.8	73.6+	3.8+	3.6
0.05% Terramycin	14	102	157	10.3	0.6	1.8	20.4	1.9	2.1	57.4+	5.9+	2.3
Intact Cotton Rats												
None	11	33	36	6.9	1.1	3.0	13.6	2.5	3.0	40.3+	8.1+	2.6
0.05% Streptomycin	11	38	34	2.4	0.7	0.3	4.7	1.6	0.3	13.5+	5.9+	0.6
0.05% Terramycin	8	26	29	6.2	1.8	2.0	12.2	4.0	1.7	33.8+	8.1+	2.0

* SEM -- Standard Error of Mean

† CR -- Critical Ratio

The results of Experiment 9 are depicted in Table VI. The intact white rats which were fed levels of 1.0 and 2.0 per cent indole grew somewhat less well than those receiving the 0.5 per cent indole and the unsupplemented ration. This approached severe proportions in the 2.0 per cent supplement. Among the cotton rats, the toxic influence upon the well-being of the animals

TABLE VI

THE INFLUENCE OF INDOLE SUPPLEMENTS ON THE DENTAL CARIES INCIDENCE OF INTACT WHITE RATS AND COTTON RATS

Ration Supplement	No. of Rats	Total Increment in Body Wt. ♀ gm.	Total Increment in Body Wt. ♂ gm.	Number of Carious Teeth Avg.	SEM*	CR†	Number of Carious Lesions Avg.	SEM*	CR†	Extent of Carious Lesions Avg.	SEM*	CR†
White Rats												
None	19	122	245	3.1	0.5		13.8	1.2		34.2+	4.2+	
0.5 % Indole	11	137	254	5.1	0.8	2.8	6.7	1.4	3.8	13.5+	2.5+	4.2
1.0 % Indole	20	118	170	6.9	0.6	1.5	9.5	1.1	2.6	18.1+	2.6+	3.3
2.0 % Indole	19	90	148	7.8	0.4	0.5	13.1	1.0	0.4	31.3+	3.1+	0.6
Cotton Rats												
None	6		60	10.8	0.7	0.1	24.8	4.1	0.0	79.8+	16.1+	0.2
0.5 % Indole	7	57	59	10.7	0.6	0.4	24.6	3.0	0.6	76.0+	15.7+	1.0
1.0 % Indole	5	28	66	10.4	0.7	0.3	22.0	1.9	0.7	59.6+	11.8+	0.8

* SEM--Standard Error of Mean

† CR--Critical Ratio

was sufficiently great that the 2.0 per cent supplement of indole was fatal to all animals in the group. Supplements of 0.5 per cent indole and 1.0 per cent indole caused statistically significant reductions in dental caries incidence among the white rats. At the 2.0 per cent indole supplement level for white rats, there was no significant reduction in any of the 3 criteria by which the dental caries incidence is appraised. This is an unusual finding and one that is inexplicable on the basis of the present data. It is noteworthy that the lesions among the animals which received 2.0 per cent indole in the diet were stained intensely blue and were extremely resistant to pressure and had little resemblance to carious lesions as ordinarily seen in this species. In many ways it appeared that there had been a penetration of the tooth substance by this highly colored material possibly even without carious lesion formation. The cotton rats which received the 0.5 per cent indole had a dental caries incidence which was almost identical with that of the control animals. The rats in the 1.0 per cent indole group had a dental caries incidence which was slightly reduced from that of the controls, but by an amount which was not statistically significant.

TABLE VII
THE INFLUENCE OF PANTHENOL IN THE DIET AND IN THE DRINKING WATER ON THE DENTAL CARIES INCIDENCE IN INTACT AND SIALO-ADENECTOMIZED WHITE RATS

Supplements	No. of Rats	Total Increment in Body Wt. ♀ gm.	Total Increment in Body Wt. ♂ gm.	Number of Carious Teeth Avg.	Number of Carious Teeth SEM*	Number of Carious Teeth CR†	Number of Carious Lesions Avg.	Number of Carious Lesions SEM*	Number of Carious Lesions CR†	Extent of Carious Lesions Avg.	Extent of Carious Lesions SEM*	Extent of Carious Lesions CR†
Intact White Rats												
20 mg. Calcium Pantothenate / kg. Diet	8	130	191	3.5	0.5		3.9	0.5		6.4+	1.4+	
300 mg. Panthenol/kg. Diet	7	160	219	3.4	0.9	1.0	4.1	1.3	0.1	8.0+	3.8+	0.4
150 mg. Panthenol / 1. Water	9	156	215	4.4	0.8	1.0	5.4	1.4	1.0	11.7+	4.3+	1.2
300 mg. Panthenol/ kg. Diet & 150 mg. Panthenol/ 1. Water	7	148	250	5.0	0.6	1.9	6.0	1.0	1.9	10.7+	2.3+	1.6
Sialo-adenectomized White Rats												
20 mg. Calcium Pantothenate / kg. Diet	7	182	243	12.0	0.4		24.9	0.8		96.4+	7.3+	
300 mg. Panthenol/ kg. Diet	6	158		12.0	0.0	0.0	26.8	0.6	1.9	107.5+	13.1+	0.7
150 mg. Panthenol / 1. Water	5	133	141	10.8	0.4	2.7	24.4+	1.5	0.3	97.2+	11.3+	0.1
300 mg. Panthenol/ kg. Diet & 150 mg. Panthenol/ 1. Water	6	153	263	12.0	0.0	0.0	26.5	0.9	1.2	100.5+	9.6+	0.2

* SEM -- Standard Error of Mean
† CR -- Critical Ratio

The results of Experiment 10 are reported in Table VII. The intact rat which received Panthenol in the diet or in the water appeared to have slightly higher growth increments during the experimental period than did the animals in the control group which received calcium pantothenate. The animals in

the 3 Panthenol groups routinely had slightly higher incidences of dental caries with the highest rate being noticed in the group where Panthenol was supplied both in the diet and in the drinking water, although there was a trend toward an increased dental caries incidence, these increases were not statistically significant. Among the sialo-adenectomized white rats the same trend toward an increase in body weight was not observed among the animals receiving Panthenol. In so far as the females were concerned, their weight was slightly less than for the control animals which received calcium pantothenate. In this sialo-adenectomized series there was no significant increase in the dental caries incidence of the animals which received Panthenol.

The amounts of Panthenol, bacitracin, Aureomycin, sodium fluoride, streptomycin, penicillin, subtilin, menadione, Chloromycetin, and tyrothricin required to produce an 80 per cent inhibition of acid production by a strain of *L. acidophilus* isolated from the oral cavity of a rat and another strain isolated from human saliva are presented in Table VIII. It is obvious from inspection of these data that the amounts of these compounds required for this degree of inhibition of acid production in an in vitro circumstance vary tremendously, from as little as 0.009 mg. per tube for tyrothricin with the human strain up to 23 mg. per tube for Panthenol with the rat strain. However, these results bear little, if any, resemblance to the degree of effectiveness of the several materials when fed individually in the diets of caries-susceptible rodents. Whether these microorganisms after many transfers bear any close metabolic resemblance to the original isolates may be open to question. In any case, in their present form and under the circumstances of this test, this series of evaluations on the inhibition of acid production serves no useful purpose in the prediction of the extent that a given material is able to influence caries initiation and progression in the test animal.

TABLE VIII

RELATIVE LEVELS OF COMPOUNDS REQUIRED TO PRODUCE AN 80 PER CENT INHIBITION OF ACID PRODUCTION BY A RAT AND A HUMAN STRAIN OF *Lactobacillus acidophilus*

COMPOUNDS	RAT STRAIN MG./10 ML. MEDIA	HUMAN STRAIN MG./10 ML. MEDIA
Panthenol	23	16
Bacitracin	13	10
Aureomycin	12	9
Sodium fluoride	2.4	0.25
Streptomycin	1.6	1.5
Penicillin	0.8	3.0
Subtilin	0.26	0.20
Menadione	0.12	0.44
Chloromycetin	0.05	0.07
Tyrothricin		0.009

By and large, similar levels of a compound were required to give an 80 per cent inhibition of acid production by the 2 strains. However, in the case of penicillin and menadione, approximately 4 times as much of each was required to inhibit acid production by the human strain as by the rat strain. But the most interesting observations in this in vitro series of trials was the tenfold

greater amount of sodium fluoride required to inhibit acid production by the rat strain than by the human strain. It would be interesting to know whether or not this difference would be consistent for a variety of strains of *L. acidophilus* from the rat and from man. If there is a consistent difference, this observation might help to explain the higher levels of fluoride required to inhibit caries production in the rat. It would seem more likely that this was a happenstance, occurring without broader implication in these two microorganisms, that may not have been representative of the oral flora of the rat and man.

DISCUSSION

The results of the present series of animal investigations show that caries was inhibited in varying degrees by the compounds tested. Of these the supplement of 0.05 per cent penicillin to the diet was clearly the most effective for the intact cotton rat and for the intact and sialo-adenectomized white rat. Concentrations of penicillin as low as 0.025 per cent and 0.01 per cent also gave highly significant decreases in decay. Aureomycin, and Aureomycin as an integral component of the APF concentrate, Chloromycetin, streptomycin, Terramycin, and indole were moderately effective in reducing the incidence of dental caries. Panthenol, tyrothricin, subtilin, and bacitracin appeared to be only slightly effective or were completely ineffective under the circumstances of these trials.

In all cases where the influence of a compound was compared in intact rats and in sialo-adenectomized rats, the degree of inhibition produced by a given level of a compound was very similar for the 2 experimental situations. This generalization would indicate that, even in view of the tremendously increased tendency for carious lesions to develop after removal of the salivary glands, the caries process could be arrested by the same agents and to approximately the same percentage levels when other factors were constant. This would support the hypothesis that the use of sialo-adenectomized rats for a variety of dental research studies is justified.

Interesting species differences were observed for several of these compounds. Aureomycin, Terramycin and indole were appreciably more effective in the white rat than in the cotton rat. In contrast, penicillin and streptomycin were appreciably more effective for the cotton rat than for the white rat. The reason for these species differences at present can neither be explained with the available knowledge of the oral microflora nor of the antibiotics. For example, Aureomycin and Terramycin are closely related both as to structure and microbiologic spectra of effectiveness. Hence, their comparable inhibitory effect on caries initiation in the white rat might be expected to carry over similarly to another species of caries-susceptible rodent. Since this parallelism was not observed, does this mean that there are different oral microflora in each of the species? Moreover, it was surprising to observe that 2 antibiotics, penicillin and streptomycin, with comparatively narrow spectra that are completely opposite to each other, were able to inhibit caries to a high degree in the cotton rat and of the 2, only penicillin was significantly effective for the white rat; yet the broader spectrum antibiotics have appreciably less effect for both species of rodents.

In view of the difference in species response to various antibiotics, it comes as no surprise to find a complete lack of coordination between the in vitro trials with isolates of *L. acidophilus* from the rat and from man and the animal experiments. For example, subtilin was completely ineffective in altering the caries incidence in white rats even though as little as 0.26 mg. per tube was sufficient to produce an 80 per cent inhibition of acid production by a rat strain of *L. acidophilus*. Yet, penicillin is highly effective for the rat even though 0.8 mg. was required to inhibit 80 per cent of acid production. An even better example from an earlier paper showed that menadione was completely ineffective in altering the dental caries incidence in cotton rats though 0.12 mg. per tube was adequate to produce an 80 per cent inhibition of acid production.[3]

The present investigation tends to emphasize that unknown factors still exist with regard to the effect of antibiotics on experimental caries. In addition to the more prominent microbiologic questions to be answered, the role of certain physicochemical processes such as absorption and penetration of the dental plaque by the antibiotics, the rate of secretion of the antibiotic in the saliva, etc., must be evaluated in much greater detail before any definite conclusions can be reached.

SUMMARY

Panthenol, bacitracin, Aureomycin, an animal protein factor concentrate containing Aureomycin, streptomycin, penicillin, subtilin, Terramycin, Chloromycetin, tyrothricin, and indole were tested as additions to a cariogenic diet for their ability to alter the rate of initiation and progression of carious lesions in cotton rats, intact white rats, and desalivated white rats. Penicillin was the most effective in reducing the dental caries incidence in both species. Aureomycin, Chloromycetin, streptomycin, Terramycin, and indole were moderately effective. Panthenol, tyrothricin, subtilin, and bacitracin were only slightly effective or were completely ineffective.

Species differences between the cotton rat and the white rat were observed for the degree of influence upon caries incidence produced by penicillin, Terramycin, indole, Aureomycin, and streptomycin.

No correlation was observed between simple in vitro tests with these antibiotics and their effectiveness in animal trials.

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ANTIGENIC ANALYSIS OF STRAINS OF HETEROFERMENTATIVE LACTOBACILLI OF HUMAN ORAL ORIGIN

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LACTOBACILLI isolated from human saliva or from tooth and other surfaces in the oral cavity may be classified among the homofermentative and heterofermentative groups described in Bergey's Manual.[1] The basis for this separation is the relative amount of lactic acid among the by-products. Homofermenters produce only traces of by-products other than lactic acid, whereas heterofermentors produce considerable amounts of other by-products (carbon dioxide, alcohols, acetic acid, and others). Species designation within each of these groups is dependent on optimum growth temperature, the optical rotation of the lactic acid, the fermentation of various chemicals and, recently, certain nutritional requirements.[2, 3]

The frequency of various species of lactobacilli in the salivas of human beings has been reported by Tilden and Svec, and Rogosa, Wiseman, Mitchell, Disraely, and Beaman.[3] These workers indicated that homofermenters occurred in the samples more frequently than did the heterofermenters but, in many instances, both kinds of lactobacilli were present in the same sample. Preliminary observations in our laboratories have indicated that when colony counts were made for single saliva samples the relative number of homofermenters was usually higher than for heterofermenters. This proportion was constant regardless of the total number of colonies. In view of the fact that most of the above observations were made on saliva from "caries-susceptible" human beings, it is interesting to consider a report by Clement and Rae.[5] They cultured the saliva from "caries-resistant" children in South Africa for lactobacilli and found that almost all cultures contained only "weak" acid formers. This characteristic and others, which Clement and Rae described, suggested that the oral environments of these human beings were favorable largely to heterofermenters.

The lactobacillus population is only a small fraction of the oral biota. Thus, the observed differences among human beings, as to (1) presence or absence of lactobacilli and (2) the variety of kinds, raise some interesting ecologic considerations. What oral conditions may regulate the lactobacillus population (either limiting it or being stimulatory)? What factors in the oral cavity are

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associated with the predominance of homofermenters over heterofermenters and vice versa? Do these conditions and proportions affect the lactobacillus populations in the intestinal tract? Satisfactory answers to such questions are not available. Harrison and Opal[6] made an important beginning by using serologic tests to identify oral strains of lactobacilli in the feces of infants. The antigenic analysis of many homofermentative strains[7] gives an opportunity to use antigenically marked strains in such studies. In view of the incidence of heterofermentative strains it has become essential to determine whether antigenic differences can be detected among these lactobacilli.

This report is concerned with a study of the antigenic relationships among a limited number of heterofermentative lactobacilli. Relationships with various other antigenic components of lactobacilli were studied by means of agglutination tests with adsorbed and unadsorbed antisera for the various strains.

MATERIALS AND METHODS

Cultures.—Human saliva, obtained from that collected for the Caries Control Laboratory, was diluted in broth and inoculated or streaked on the surface of tomato juice agar,[8] pH 5.0, and incubated aerobically at 37° C. for 72 hours. Colonies arising on the medium, considered to be lactobacilli, were selected at random for description and transfer to a modification of the SL medium (acetic acid and agar were omitted) of Rogosa, Mitchell, and Wiseman.[9] The inoculated medium was incubated at 37° C. for 24 hours. Gas production was tested in a modification (0.5 per cent yeast extract was substituted for the gelatin) of an agar medium described by McLaughlin.[10] The medium was liquefied and cooled to 47° C., inoculated with 0.1 ml. of growth suspension from the above broth culture, then solidified by cooling rapidly. Sterile mineral oil was added to a depth of about 1 cm. on the surface of the medium before incubating at 37° C. Five such tubes were prepared from each broth tube. Splitting of the agar in *all* of the tubes was required for presumptive evidence for heterofermentative lactobacilli. The cultures were replated and single colonies tested for gas production two more times before seed cultures were prepared for production of cells for serologic studies.

Stock cultures were made from the quantity production and were maintained in duplicate on slanted SL medium (modified as above except for agar) and litmus milk. Strains were preserved also by lyophilization, then stored at 5° C. A total of 49 colonies was selected from the cultures of saliva; of these, 27 were found to be heterofermenters. Strains were selected at random for identification physiologically[3] and for the serologic studies. Using American Type Culture Collection (ATCC) strains of *L. fermenti* (#9338), *L. buchneri* (#11577), and *L. cellobiosus* (#11739) as controls, strains isolated from saliva were identified as follows: #14-1 and #126-2-1 as *L. fermenti;* #90-2 and #835-1-3* as *L. buchneri;* and #127 as a variant of *L. buchneri.*

*Studies of this strain were not possible because antibodies could not be induced in the experimental animals.

Vaccines and Agglutinogens.—Seed cultures were made from our isolates and also #9338 (ATCC) to inoculate quantities of the liquid SL medium at pH 6.0 to produce enough growth for all of the serologic studies. Cells were removed by centrifugation, after 3 to 4 days' incubation at 37° C., then were washed 3 times with sterile 0.85 per cent NaCl in distilled water. The packed cells, about 10 ml., were suspended in enough sterile 0.85 per cent NaCl solution to make a final volume of 100 ml. This was termed a "concentrated" suspension; a "dilute" suspension was prepared by adding sterile 0.85 per cent NaCl solution until a reading of 280 on a Klett-Summerson colorimeter was obtained using a 470 mμ filter. "Concentrated" and "dilute" suspensions of the various strains were transferred to sterile, rubber-stoppered bottles containing sterile glass beads to facilitate resuspension. The bottles were placed in a water bath at 60° C. for about 2 hours in order to inactivate the bacteria. After cooling and testing for microbial viability, the bottles were stored at 5° C. until used for animal injections. Suspensions for agglutinating antigens were prepared in the same way as just described except that, instead of heating, phenol was added as a preservative, final concentration 0.2 per cent by volume.

Antisera.—Male rabbits, weighing about 2.5 Kg., were kept in quarantine for 2 weeks before inoculations were started. Serum was collected and tested for agglutinins for the various strains to be used in the tests. Two rabbits were injected with vaccine for each strain. One or the other of the following inoculation schedules was used:

a. One milliliter of "concentrated" vaccine was mixed with 4 ml. of sterile 5 per cent sodium alginate solution and injected intraperitoneally; using the same syringe and needle, 2.5 ml. of sterile 1 per cent calcium chloride in distilled water was injected into the same site.[11] Intravenous inoculation with 1 ml. of "dilute" vaccine was started 3 weeks later and repeated every fourth day until 3 ml. had been injected. A preliminary bleeding was performed 10 days after the last intravenous injection; if the titer was less than 3 plus agglutination in a serum dilution of 1:1280, the intravenous series was repeated. Usually a satisfactory titer was obtained without repeating the intravenous injections.

b. Intravenous injections of 0.2 ml. of "concentrated" vaccine were made on 4 successive days in the first week. In the second week, 0.5 ml. was injected intravenously on 4 consecutive days; then 1 ml. on 4 consecutive days in the third week. Preliminary bleeding was done 1 week after the last injection, if the titer was unsatisfactory, inoculations similar to those in the third week were repeated. A satisfactory titer could not be attained without repeating the intravenous injections of 1.0 ml.

New animals were used if neither of the above schedules produced antiserum with the desired titer. Animals were bled from the ear vein within 24 to 48 hours after agglutination tests had indicated that the serum contained agglutinins in the desired concentration. Phenol was added to all sera to a final concentration of 0.2 per cent by volume.

Agglutination and Agglutinin-Adsorption.—Agglutinating antigen was prepared from phenol-preserved suspensions of the desired strains by twice filtering an aliquot of the suspension through cotton, using negative pressure. The resulting suspension was diluted with 0.85 per cent NaCl solution until a

turbidity of 145 on a Klett-Summerson colorimeter was obtained using a 470 mμ filter. An equal volume of the antigen was added to an equal volume of diluted serum, mixed and placed in a water bath at 37° C. for 2 hours, then at 5° C. overnight. The final dilutions of serum ranged between 1:20 and 1:5120. Readings were made against a dark background: 4 represented complete, 3 incomplete, and 2 partial agglutination. The titer of the serum was the highest dilution showing an agglutination of 3 or better.

For adsorption of agglutinins, a 0.5 ml. volume of centrifuged, washed cells was suspended in 10 ml. of a 1:10 dilution of the serum being adsorbed in a sterile test tube, and the tube was closed with a sterile rubber stopper. The tube was placed on a mechanical device which continually mixed the contents at 37° C. for 2 hours. The cells were removed by centrifugation and the above procedure repeated using fresh cells on 2 more occasions. Most agglutinins were removed after 3 adsorptions, but, occasionally, 4 were required. The adsorbed serum was preserved by adding phenol to 0.2 per cent by volume and stored at 5° C.

RESULTS

The relationships among the 5 strains of heterofermentative lactobacilli are indicated in the results of homologous and heterologous agglutination tests in Table I. Strain #126-2-1 is related antigenically to all of the other strains, its cells are agglutinated by all antisera in titers of 1:320 or higher. Antiserum #126-2-1 agglutinates all heterologous cells, the lowest titer being 1:160. Antiserum #9338 agglutinates antigen #90-2 and also #14-1; the titers are lower than the homologous or the agglutination of #126-2-1 cells. The cells of #127 are not agglutinated. Antiserum #14-1 agglutinates homologous cells and those of #126-2-1 in high titer. The cells of #127 and #9338 are agglutinated in low titer. Antiserum #127 agglutinates homologous cells in high titer; agglutination also is observed with #126-2-1 and #14-1 cells but in low titer. These data seem to indicate one relationship between #9338, #90-2, and #126-2-1; a second relationship between #9338 and #14-1; and a third relationship between #126-2-1, #14-1, and #127.

TABLE I

AGGLUTINATION RELATIONSHIPS OF 5 STRAINS OF HETEROFERMENTATIVE LACTOBACILLI*

ANTISERA STRAIN NUMBER	CULTURE SUSPENSIONS				
	#9338	#90-2	#126-2-1	#14-1	#127
9338 (ATCC *L. fermenti*)	1280†	320	640	160	0
90-2 (*L. buchneri*)	160	1280	640	0	0
126-2-1 (*L. fermenti*)	1280	640	5120	640	160
14-1 (*L. fermenti*)	80	0	1280	2560	160
127 (*L. buchneri*)	0	0	320	160	1280

*Agglutination tests with sera from animals prior to preparation of antisera were negative in lowest dilutions tested (1:20).

†Figures represent reciprocals of highest antiserum dilutions in which at least a 3+ agglutination occurred. All controls were negative.

Agglutinin-adsorption tests were carried out to analyze the relationships found by the agglutination tests. All of the antisera were adsorbed with homologous organisms; in each instance there was no agglutination in the lowest dilution of the serum tested (1:20). All antisera, prepared against the strains listed in Table I, were adsorbed with each heterologous strain and then were used in agglutination tests. The results of the agglutinin-adsorption tests with the various strains are presented in Table II. The data from agglutination reactions for each antiserum prior to adsorption are repeated from Table I to help follow the analysis. Complete mirror adsorptions (i.e., each antiserum was adsorbed with each strain) were carried out, but if results of agglutination tests after adsorption with 1 strain were found to be the same as after agglutination tests with another strain, only 1 set of these results was included. As the multiplicity of components, revealed by the adsorption tests, has complicated the analysis and the preparation of monospecific antisera, Table II will be discussed according to each strain rather than according to component. Assignment of numbers to the antigenic components among these lactobacilli will follow the scheme introduced by Williams, Norris, and György.[12] The lactobacilli used in this work are considered to fall in the group designated by IIb (I = homofermenters; II = heterofermenters; IIa = branched forms; IIb = unbranched forms), and since the arabic numeral one has already been used,[12] the first major component (agglutinating titer over 1:1000) among these strains is given the arabic numeral *2*.

TABLE II
AGGLUTININ ADSORPTION TESTS

ANTISERUM	ADSORBED WITH ANTIGEN	CULTURE SUSPENSIONS					ANTIBODIES IN ADSORBED ANTISERA*
		#9338	#90-2	#126-2-1	#14-1	#127	IIb
#9338*	unadsorbed	1280†	320	640	160	0	
	90-2	640	0	320	160	0	2,3 (m-1)
	126-2-1	1280	320	0	160	0	2,3 (m-1)
	14-1	320	320	160	0	0	2,3
	90-2 + 126-2-1	160	0	0	0	0	2
#90-2	unadsorbed	160	1280	640	0	0	
	9338	0	320	0	0	0	4
	126-2-1	0	640	0	0	0	4
#126-2-1	unadsorbed	1280	640	5120	640	160	
	9338	0	0	2560	320	160	5,6 (m-2)
	90-2	640	0	2560	640	160	3,5,6 (m-2
	14-1	80	640	2560	0	0	3,5
	127	1280	640	5120	640	0	3,5,6
	9338 + 14-1	0	0	1280	0	0	5
#14-1	unadsorbed	80	0	1280	2560	160	
	9338	0	0	320	640	80	6 (m-2)
	127	40	0	320	640	0	6 (m-1)
#127	unadsorbed	0	0	320	160	1280	
	126-2-1	0	0	0	0	1280	7

*Each antiserum gave a negative agglutination test after adsorption with homologous bacteria.

†Figures represent reciprocals of highest dilution in which a 3 agglutination occurred.

Strain #9338 (*L. fermenti,* ATCC).—Adsorption of the antiserum with cells of strain #90-2, #126-2-1, or #14-1 removed agglutinins for each strain but not for the homologous or other strains. This seemed to indicate that separate components were involved. However, study of agglutinin-adsorption tests using antiserum #90-2 or #126-2-1 showed the relationships to be more complex. First, it is noted that when #9338 antiserum is adsorbed with strain #90-2 and also #126-2-1, only the #9338 cells were agglutinated by the adsorbed antiserum. It is concluded that strain #9338 contains a component labeled *2.* It is proposed in addition that the strain has a component labeled *3,* which may also be revealed in strains #90-2 and #126-2-1; however, evidence will be presented which suggests that the relative amount of this component in these strains is lower than that in strain #9338. Such differences in the relative amount of this antigen could account for the removal of agglutinins for each adsorbing strain (#90-2 or #126-2-1) without completely removing agglutinins for other cells. The relationship between strains #9338 and #14-1 is represented by m-1 to indicate the existence of one of two "minor" components among these 5 strains.

Strain #90-2 (*L. buchneri*).—Strains #9338 and #126-2-1 agglutinated in the antiserum but #127 and #14-1 did not. When the antiserum was adsorbed with either #9338 or #126-2-1, agglutinins for the other, but not the homologous were removed. Furthermore, adsorption of the same batch of antiserum with both strains failed to remove the homologous agglutinins, thus component *4* is assigned to strain #90-2 along with component *3.* These data give more support to the assumption that there is a common antigen between these 3 strains. Adsorption with strain #127 or #14-1 did not alter the agglutinin titers for strains #90-2, #9338, and #126-2-1 in #90-2 antiserum.

Strain #126-2-1 (*L. fermenti*).—The antiserum for this strain contains agglutinins for all of the heterologous strains. Adsorption with strain #9338 removes agglutinins for itself and also strain #90-2. This is probably due to the common existence of component *3* among these strains. The concentration of other agglutinins is not appreciably affected. Adsorption with strain #90-2, removes agglutinins for itself but not for other strains. Note that cells of strain #9338 are agglutinated after adsorption with strain #90-2, presumably because of component *3* in the antiserum. This seems to support the proposal that component *3* exists in different proportions in the different strains; #9338 and #126-2-1 contain more than #90-2, and #9338 contains more than #126-2-1. Distribution of antigen in different amounts in different strains of the same kinds of bacteria has been observed among the Brucella.[13]

Adsorption of antiserum #126-2-1 with cells of strain #14-1 removes agglutinins for those cells as well as for strain #127, and the titer for strain #9338 is greatly reduced. These results indicate that antiserum #126-2-1 has specific agglutinins for cells #14-1. Adsorption with cells of strain #127 removes agglutinins for itself only; these agglutinins are represented by m-2 since they are considered minor. Adsorption of antiserum #126-2-1 with

cells of strains #9338 and #14-1 removes all but the homologous agglutinins. Thus, component *5* is assigned for the homologous component and component *6* for the relationship with strain #14-1.

Strain #14-1 (*L. fermenti*).—Adsorption of the antiserum with cells of strain #9338 removed agglutins for those cells and some agglutinins for other strains as indicated by the reduced titers. Adsorption with strain #127 removed agglutinins for only that strain, whereas adsorption with cells of strain #126-2-1 removed all agglutinins. Component *6* is assigned to strain #14-1 as the major agglutinin and agglutinogen. It is proposed, because of the interrelationships with strains #9338 and #127, that strain #14-1 contains the minor components m-1 and m-2.

Strain #127 (*L. buchneri*).—Adsorption of the antiserum with the cells of either strain #126-2-1 or #14-1 removed all agglutinins for each of these strains but agglutinins for strain #127 were not diminished. Component *7* is assigned to the major agglutinins in this antiserum. Adsorption of the antiserum with the cells of either strain #9338 or #90-2 did not diminish the agglutinins for the other strains, thus the minor agglutinins are probably m-2.

A recent report by Sharpe[14] concerning the use of crude extracts, prepared by the Lancefield[15] method, stimulated the preparation of such extracts from our cultures. Extracts were prepared from washed cells and layered on undiluted and unadsorbed antiserum. A precipitate forming at the interface within 30 minutes at room temperature (25 to 30° C.) was considered a positive reaction. The results shown in Table III verify, to some extent, the findings from the antigenic analysis, and also support the contention of differences in amount of the substance which is presumed to relate strains #9338, #90-2, and #126-2-1 to one another. If #9338 contains the greatest amount, it would be expected that extracts of #90-2 and #126-2-1 would react with the antiserum, however it also would be expected that extracts would not react with antiserums containing lesser amounts. Note that in #90-2 antiserum, #9338 reacts very weakly and #126-2-1 is negative. Also in #126-2-1 antiserum, #9338 reacts, but #90-2 does not. Only one major relationship (between strains #90-2 and #126-2-1) is not indicated by the precipitin test; and nearly all of the cross reactions observed in agglutination tests, accounted for by minor components, are eliminated. Extract from strain #14-1 reacted in antiserum

TABLE III
PRECIPITATION TESTS (RING) USING CRUDE EXTRACTS

UNDILUTED ANTISERA	EXTRACTS FROM CELLS OF STRAIN				
	#9338	#90-2	#126-2-1	#14-1	#127
#9338	+	+	+	+	0
#90-2	±	+	0	0	0
#126-2-1	+	0	+	+	0
#14-1	0	0	+	+	0
#127	0	0	0	0	+

+ = precipitate at interface between serum and extract.
± = questionable precipitate.
0 = no precipitate.

#9338 but extract from #9338 was not precipitated by antiserum #14-1. It will be noted in Table I that the cells of strain #14-1 and strain #9338 were agglutinated in relatively low titer by the respective heterologous antisera.

The major components among the 5 strains included in this study are shown in Table IV. Antisera containing monospecific agglutinins could be obtained for all components except component *3* and the minor components. Strain #126-2-1 contains three major components; strains #9338 and #90-2 contain 2; and strains #127 and #14-1 each have 1.

TABLE IV
ANTISERA CONTAINING SPECIFIC ANTIBODIES

STRAIN NUMBER	ANTIBODIES IN ADSORBED SERA					
	IIb·2*	IIb·3,5	IIb·4	IIb·5	IIb·6	IIb·7
9338	+†	+	0	0	0	0
90-2	0	+	+	0	0	0
126-2-1	0	+	0	+	+	0
14-1	0	0	0	0	+	0
127	0	0	0	0	0	+
126-2-2	0	+	0	+	+	0
126-1	0	+	0	+	+	0
90-1	0	0	+	0	0	0
90-3	0	0	+	0	0	0
14-2	0	0	0	0	+	0
5A	+	0	0	0	0	0
5B	+	0	0	0	0	0
1708	0	0	0	0	0	+
1736	0	0	0	0	0	+
10B	0	0	0	0	0	+
12	0	0	0	0	0	+
11	0	0	0	0	0	+
4B	0	0	0	0	0	+
6A	0	0	0	0	0	+
ATCC 367 *L. brevis*	+	0	0	0	0	0

*IIb·2 = # 9338 antiserum adsorbed with #90-2 and #126-2-1 cells.
IIb·3, 5 = #126-2-1 antiserum adsorbed with #14-1 cells.
IIb·4 = #90-2 antiserum adsorbed with #126-2-1 cells.
IIb·5 = #126-2-1 antiserum adsorbed with #9338 and #14-1 cells.
IIb·6 = #14-1 antiserum adsorbed with #9338 and #127 cells.
IIb·7 = #127 antiserum adsorbed with #126-2-1 cells.

†Each + sign indicates 3+ agglutination in dilution of 1:160 or more of the adsorbed serum.

The preparation by agglutinin-adsorption of antisera which contained specific antibodies gave an opportunity to study the serologic relationships with physiologically similar and dissimilar lactobacilli (see Table IV). Of 23 heterofermentative strains isolated from saliva for this study, 14 (#126-2-2 to #6A) were agglutinated in 1 or more of the antisera. Half of these were agglutinated by the antiserum containing component IIb·7, and only 2 of the 14 reacted with more than 1 antiserum. Strains of heterofermentative lactobacilli, *L. brevis, L. buchneri, L. fermenti,* and *L. cellobiosus* nov. spec., obtained from the ATCC, were also tested, but only 1 (*L. brevis* ATCC 367) of 8 different strains was agglutinated.

Strains of lactobacilli from the homofermentative group (mostly *L. casei*) and other heterofermentative strains (*L. bifidus* and *L. parabifidus*) were tested

in the antisera prepared in this study before and after adsorption. Antisera for the heterofermentative strains were usually more reactive before than after adsorption. At least 6 of the homofermentative strains were agglutinated in low titer (1:160 or lower) by 1 or more of the heterofermentative antisera (IIb·2 through 7). Only a few of the heterofermentative strains were agglutinated (also in low titer) in the homofermentative antisera (I-1 through 9). None of the homofermenters or heterofermenters was agglutinated in the other antisera when these antisera had been adsorbed, leaving only specific antibodies. Thus, it appears that these kinds of lactobacilli may be related through common minor antigens; but by using antisera containing specific antibodies it is possible to differentiate the various physiologically unrelated strains of lactobacilli.

DISCUSSION

The serologic relationships among the heterofermentative lactobacilli included in this work may be accounted for by major and minor antigens in the bacterial cells. The relationship between strains #9338, #90-2, and #126-2-1 is apparently due to a major antigen which was present in different amounts in these strains. Although an unequal distribution of an antigen has been known to exist in the Brucella, this has not been observed previously among the lactobacilli. As has been reported by others,[13] use of adjusted adsorbing doses of the bacterial cells in the antisera makes it possible to determine which antisera contain relatively great or small amounts of the antibody. There is indirect evidence also for unequal distribution of minor antigens among certain of the strains. Minor agglutinins have been recognized previously in antigenic analyses of lactobacilli.[16, 17] Their presence in antisera was believed due to long vaccination programs (thus involving large amounts of the bacteria) required to produce satisfactory antisera. However, it was also noted that the sera of the rabbits before injection may contain antibody.[5] In this work, even though none of the rabbit sera contained agglutinins for the cells to be inoculated, minor agglutinins appeared in the antisera regardless of the time or amount of vaccine required to produce a satisfactory (titer 1:1280) antiserum.

Recently, Sharpe[14] reported studies on the precipitation (ring test) reactions of a large collection of lactobacillus strains (including homo- and heterofermenters). The results indicated 6 serologic groups which apparently corresponded with the species names of the various strains. The author considered her results as indicating "group" relationships among lactobacilli comparable to the group relationships among the *beta* streptococci. These results, though interesting, will require confirmation because in some respects they are at variance with the findings of others. Orland[17] reported no correlation between general fermentative capacity (a criterion for species designation) and serologic reactivity among homofermenters. In the study reported here several kinds of heterofermentative lactobacilli, 3 strains of *L. fermenti* and 2 of *L. buchneri*, were studied physiologically and serologically. There is no indication that the observed serologic reactions (agglutination and precipitation) separate the strains into groups such as those described by Sharpe.[14] The strains identified

as *L. buchneri* were more closely related serologically to the strains of *L. fermenti* than to one another. It was also noted that *L. brevis* ATCC #367 was agglutinated by *L. fermenti* ATCC #9338 antiserum, but no others. Thus, there seems to be no relation between general fermentative patterns and serologic activity of heterofermentative lactobacilli.

Detailed study of extracts of lactobacilli may yield information as to whether different antigens in lactobacilli may be responsible for the "type" and "group" reactions suggested by Sharpe.[14] Preliminary data on the serologic reactions of purified carbohydrate and protein extracts of lactobacilli confirm some of her observations. Some strains could be differentiated by using carbohydrate extracts in precipitation (ring) tests, but protein extracts of the same strains cross reacted in precipitation (ring) tests using the same antisera.* If these results are supported by further study, it may be that a carbohydrate is responsible for the "type" reactions and a protein is responsible for the "group" reactions of lactobacilli. Such a finding would be opposite to the suggestion by Sharpe[14] that the grouping among lactobacilli is based on antigenic relationships similar to those for Group A streptococci.

Characteristics such as cellular and colonial morphology, physiologic activity, and nutritive requirements have been fairly well characterized for the various strains of lactobacilli, but their antigenic structure is not clearly defined. It is apparent from the agglutination studies reported here that the 2 major groups of lactobacilli (homo- and heterofermenters) may possess common minor antigens but not major antigens. The latter is supported by Orland[16] who reported no cross reactions after testing the cells of heterofermenters as well as of the other genera in the family *Lactobacteriaceae* in adsorbed homofermentative antisera (containing specific antibodies). We have also observed that when crude extracts of homo- or heterofermenters are tested with the respective antisera (adsorbed or unadsorbed) there are no cross reactions. This seems to indicate either that the extracts do not contain minor antigens, or there is an insufficient concentration to react with the antisera. Harrison[18] reported that 2 groups of oral lactobacilli which differed in their capacity to ferment sorbitol, mannitol, and milk could also be distinguished by precipitation tests using carbohydrate extracts. It is not clear whether homofermentative and heterofermentative lactobacilli were involved, however, the characteristics of 1 of the groups strongly suggests that it contained predominantly heterofermentative lactobacilli. Until there is better understanding of the antigenic relationships, it is desirable to retain the distinction between the various strains as indicated by morphologic and physiologic characteristics. Thus, the use of species names to define specific serologic groups seems premature.

SUMMARY

1. An antigenic analysis using agglutination and agglutinin-adsorption tests of 5 strains of heterofermentative lactobacilli indicated the presence of 6 major antigens labeled II·2,3,4,5,6, and 7. Evidence was obtained for at least 2 minor antigens, m-1 and m-2.

*These observations were made by Sterling W. Jackson in this laboratory.

2. Antigenic component IIb·3 was present in strains #9338, #90-2, and #126-2-1 in different amounts, accounting for differences in serologic reactions after adsorption of respective antisera.

3. A total of 15 of 29 strains of heterofermentative lactobacilli was agglutinated by 1 or more of the antisera containing monospecific antibodies.

4. Cross reactions between the strains under study and the antisera of homofermentative lactobacilli or strains of *L. bifidus* or *L. parabifidus* (heterofermenters) were observed only in unadsorbed antisera. No cross reactions were observed when antisera containing monospecific antibodies were employed.

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EFFECT OF 2-ACETYLAMINO-1,3,4-THIADIAZOLE-5-SULFONAMIDE (DIAMOX) ON DENTAL CARIES RESISTANCE IN HAMSTERS

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FOR about 4 years (1948-1952), the hamsters used in this laboratory (and others) were highly susceptible to dental caries, induced by feeding them a high carbohydrate diet.[1-6] Then, in the course of 6 different studies involving more than 450 animals, the hamsters failed to develop sufficient caries to satisfactorily compare scores of experimental and control groups.[7-9] This unexplained resistance was noticed in other laboratories.[10]

Recently Niedermeyer, Stone, Dreizen, and Spies[11] reported that Diamox, a carbonic anhydrase inhibitor and diuretic decreased the buffer capacity, sodium, and bicarbonate content of the saliva of human beings. Also, a decrease in salivary flow and a lowered salivary pH were implied. In view of this, we felt that the drug might encourage the development of dental caries in resistant animals.

METHODS

One hundred Syrian hamsters, 20 to 40 days of age, were purchased from a source from which we previously had obtained caries-resistant animals. They were fed (ad libitum) a high carbohydrate (cariogenic) diet† which in earlier studies had been effective. After 37 days, the animals were divided into 2 groups of 20 ♂ and 20 ♀ each.

The control group remained on the caries-producing diet for an experimental period of 98 days, and the experimental group was fed the same diet with .015 per cent Diamox added. After 75 days of the experimental period, the Diamox was increased to .025 per cent for the remaining 23 days of the experiment.

The pH of saliva of 6 ♂ and 6 ♀ experimentals, and a like number of control animals was measured 3 times during the experimental period, by the use of indicator papers, or the following method. A small cotton ball, held in the restrained hamster's mouth with forceps, became soaked with saliva. This was then placed in the barrel of a tuberculin syringe, and the saliva was expressed into the container for the one-drop electrode of a Beckman Model G pH meter and measured immediately. The animals were weighed periodically and water consumption of the 2 groups was measured 4 times during the experimental period. Cursory inspections of the appearance of the urine were made.

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†Corn starch 20%; whole wheat flour 30%; whole powdered milk 30%; sucrose 15%; alfalfa 4%; sodium chloride 1%.

When the animals were sacrificed, the kidneys of representative animals were removed and prepared for microscopic study. The jaws of the animals were scored for periodontal disease at the time of sacrifice[12] and later they were stripped of soft tissues and scored for caries.[13]

RESULTS

The weight of both groups increased during the study; however, the experimental group gained only 11 Gm. (avg.) as compared to 30 Gm. for the controls. Fourteen animals (7 controls, 7 experimentals) contracted diarrhea, but only 5 died. The pH of the saliva of representative animals of both groups was 8 when measured in the ninth week by indicator papers. In the eleventh week the pH meter indicated the following averages: experimental ♀ 8.6; ♂ 8.7; control ♀ 8.6 and ♂ 8.6. At this time the dosage was increased. In the fourteenth week the readings were as follows: experimental ♀ 8.6; ♂ 8.6; control ♀ 8.8; ♂ 8.7.

Water consumption, measured the first, second, fourth, and thirteenth weeks, averaged .052, experimental, and .057, control, as cubic centimeter per day per gram of weight. No change in the appearance of the urine of the experimental animals was noted.

TABLE I
DENTAL CARIES SCORES

	CONTROL		EXPERIMENTAL	
No. animals	37	(19 ♀, 18 ♂)	38	(18 ♀, 20 ♂)
No. cavities	52	(0-6)*	157	(0-10)*
Area affected	40	(0-5½)	108	(0-13)
Molars affected	51	(0-5)	144	(0-8)
	♂ 1.8	Avg.	♂ 4.9	Avg.
	♀ 0.94	Avg.	♀ 2.6	Avg.
Insusceptibles	12	(9 ♀, 3 ♂)	3	(♀)

*Individual range.

The number of cavities and the number of molars affected was almost 3 times as great among the experimental animals, compared to the controls. The corresponding area affected was more than twice as great. The caries incidence of the males was considerably higher than that of the females, as has been reported frequently in previous studies. Only 3 ♀ experimental animals were insusceptible to caries as compared to 9 ♀ and 3 ♂ controls. The cavities induced by Diamox in these resistant animals approximate in number those formerly found in susceptible animals. One can only speculate as to the ability of this drug to raise caries scores in susceptible hamsters or other animals. The mechanism of this action is not apparent. The periodontal disease scores for both groups were similar. The relationship between this form of induced periodontal disease and dental caries remains intriguing and not clear. Kidney sections representative of the 2 groups revealed no consistent or remarkable differences.

SUMMARY

When 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, a diuretic, was fed to caries-resistant hamsters at the rate of .015 to .025 per cent of a caries-producing

diet, ad libitum, for 98 days, they developed nearly 3 times as much dental caries as their caries-resistant controls. The treated animals gained less weight than the controls, but no differences were detected in salivary pH or water consumption.

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A COMPARISON OF THE SNYDER COLORIMETRIC TEST WITH THE LACTOBACILLUS COUNT USING ROGOSA MEDIUM

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TWO of the more widely used of technics for evaluation of dental caries activity are the lactobacillus count and the Snyder colorimetric test which measures the acidogenic potential of the oral flora. Snyder,[3, 4] and Snyder and Clarke[5] report a close parallel between the Snyder test and the lactobacillus count. Sullivan and Storvick,[6] in a salivary analysis of 555 Oregon school children, reported a "highly significant" positive correlation between the Snyder test and the lactobacillus count using the method of Hadley. Fitzgerald,[7] using 30 samples of saliva from a group of 30 adults, showed a very high correlation between the Snyder test and the lactobacillus count, using both the lactobacillus medium of Rogosa and pH 5.0 tomato juice agar, while Rizzo and Tilden,[8] in a study of 140 orphanage children, showed a statistical comparison between the tomato agar lactobacillus count and the Snyder test. It is the purpose of this paper to compare the results obtained in a large number of controlled patients by the Snyder colorimetric test[1] and the lactobacillus count, using the Rogosa medium.[2]

Three separate samples of saliva, or a total of 3,147 samples, were collected at least 1 week apart from 1,049 Naval recruits. These tests were carried out at the United States Naval Training Center, Bainbridge, Md., from January, 1955, to January, 1956. This group had an average age of 17.5 years and included 63 individuals who were clinically free of caries experience. All of these subjects lived under the same conditions, were served the same foods, and were under rigid controls at all times. All salivary collections were made with supervision under similar conditions at 11 o'clock in the morning. The patients were instructed not to brush their teeth the morning of the collection and not to eat, drink, or smoke for at least 5 hours prior to the collection. Twenty milliliters of paraffin stimulated saliva was obtained from each subject and was placed in a refrigerated glass tube which was kept stoppered when not actually in use. The saliva was kept under refrigeration until used and the time elapsing between the collections of the saliva and the start of the tests was never more than 4 hours.

Approximately 3 to 4 ml. of whole saliva was shaken in a 5 ml. stoppered test tube for 2 minutes. Using a standard medicine dropper, 4 drops or about

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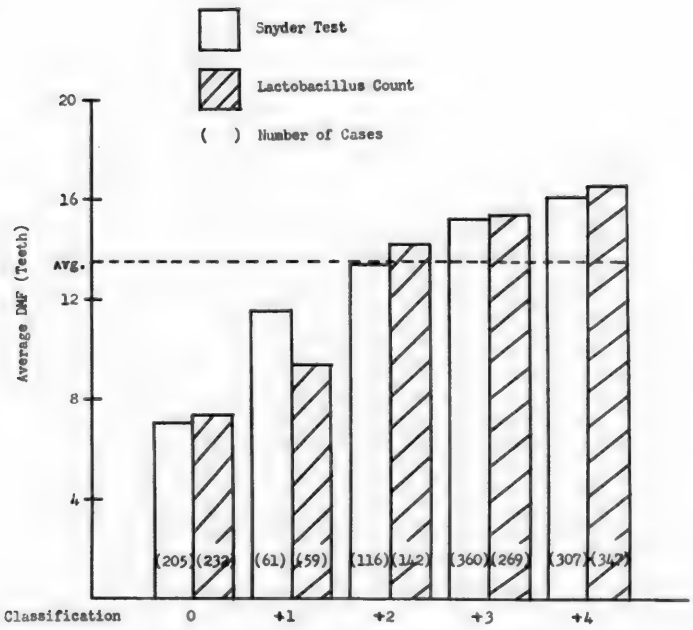

Fig. 1.—Snyder test, lactobacillus count, and average DMF (teeth). (1,049 cases.)

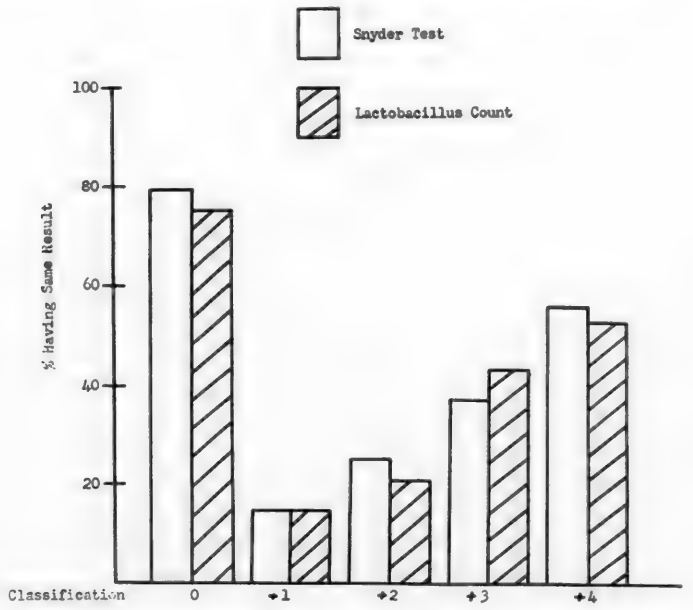

Fig. 2.—Snyder test and lactobacillus count. Per cent of each classification having the same results. (3,147 samples.)

.2 ml. of the saliva was used to inoculate approximately 5 ml. of Snyder medium. An additional 1 ml. was transferred to an 8-ounce prescription bottle containing 99 ml. of distilled water which was also shaken for 2 minutes. One milliliter of this 1:100 dilution was then transferred to a Petri dish and a pour plate made, using the Rogosa medium. The lactobacillus plates were incubated at 37° C. for 72 hours and were then counted, using a Darkfield Quebec Colony counter with a 1.5 × magnification and a Wolffhuegel counting plate. The Snyder tubes were incubated at 37° C. and read against a standard tube on a white background utilizing reflected light after 24, 48, 72, and 96 hours.

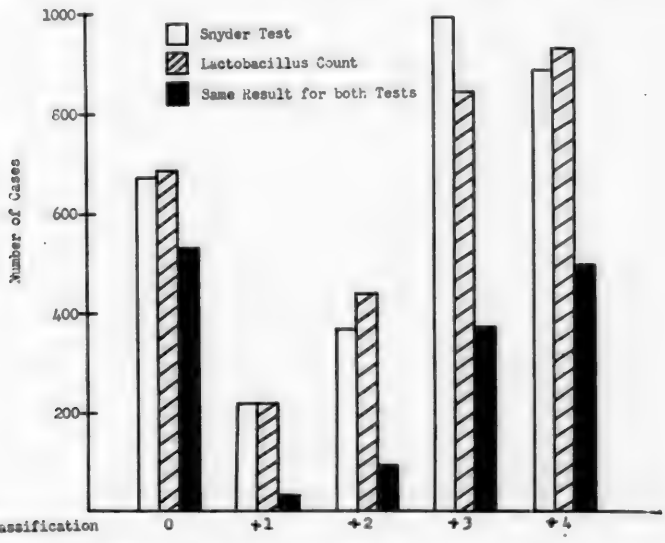

Fig. 3.—Snyder test and lactobacillus count results. (3,147 samples.)

The lactobacillus counts and the Snyder test results were graded as shown in Table I.

TABLE I

CLASSIFICATION OF LACTOBACILLUS COUNTS AND SNYDER COLORIMETRIC TESTS

CLASSIFICATION	LACTOBACILLUS COUNT NO. OF COLONIES/ML. OF SALIVA	SNYDER TEST TIME OF COMPLETE CHANGE TO YELLOW
0	0 to 199	Incomplete in 96 hours
+1	200 to 999	96 hours
+2	1000 to 9999	72 hours
+3	10000 to 99999	48 hours
+4	100000 and over	24 hours

These classifications follow the generally accepted pattern with the exception being that the Snyder tests were rated as to when a complete change to a yellow color took place rather than as to the color gradient shown at the

end of 48 hours' incubation. This was done because it was felt that a finer distinction between cases would show up in the results, especially in those subjects exhibiting slower fermentation reactions.

To evaluate the relationship of these tests to dental caries experience, the results obtained by using single samples of saliva from 1,049 different subjects were related to the number of DMF teeth. These results are summarized in Tables II, III, IV, V, and VI.

TABLE II

PER CENT OF TOTAL OF SNYDER TEST, LACTOBACILLUS COUNT, AND DMF (TEETH) (1,049 CASES)

	0 (0)	+1 (1-6)	+2 (7-12)	+3 (13-18)	+4 (19 AND OVER)
Snyder test	21.4	7.0	11.8	31.6	28.2
Lactobacillus count	20.2	9.3	13.0	26.8	29.7
DMF	6.0	13.1	22.2	33.4	25.3

TABLE III

SNYDER COLORIMETRIC TEST AND DMF (TEETH)

CLASSIFICATION	NO. OF CASES	AVERAGE DMF
0	205	7.1
+1	61	11.7
+2	116	13.4
+3	360	15.2
+4	307	16.1
Group	1,049	13.5

TABLE IV

LACTOBACILLUS COUNT AND DMF (TEETH)

CLASSIFICATION	NO. OF CASES	AVERAGE DMF
0	232	7.3
+1	59	9.5
+2	142	14.2
+3	269	15.4
+4	347	16.5
Group	1,049	13.5

TABLE V

RELATIONSHIP OF DMF (TEETH) TO SNYDER TEST AND TO LACTOBACILLUS COUNT (1,049 CASES)

DMF	% HAVING 0	% HAVING +1	% HAVING +2	% HAVING +3	% HAVING +4
		Snyder Colorimetric Test			
0	66.7	4.8	9.5	11.1	7.9
1-6	55.5	8.8	10.9	15.3	9.5
7-12	15.5	8.5	12.0	36.5	27.5
13-18	10.3	4.6	11.4	41.2	32.5
19 and over	5.7	3.7	10.2	38.5	41.9
		Lactobacillus Count			
0	73.1	6.3	7.9	6.3	6.4
1-6	62.8	12.3	9.5	8.8	6.6
7-12	20.2	8.2	16.2	27.5	27.9
13-18	8.8	3.7	14.2	32.8	40.5
19 and over	8.3	2.3	13.6	27.9	47.9

TABLE VI

RELATIONSHIP OF SNYDER TEST AND LACTOBACILLUS COUNT TO DMF (TEETH)
(1,049 CASES)

DMF	% OF 0	% OF +1	% OF +2	% OF +3	% OF +4
		Snyder Colorimetric Test			
0	20.4	4.9	5.2	1.9	1.6
1-6	37.1	19.7	12.9	5.8	4.2
7-12	17.6	32.8	24.1	23.6	20.9
13-18	17.6	26.2	34.5	40.3	37.1
19 and over	7.3	16.4	23.3	28.4	36.2
		Lactobacillus Count			
0	19.8	6.8	3.5	1.5	1.2
1-6	37.1	28.8	9.2	4.4	2.6
7-12	20.3	32.2	26.8	23.8	18.7
13-18	13.3	22.0	35.2	42.8	40.9
19 and over	9.5	10.2	25.3	27.5	36.6

It would seem quite obvious that while there is a good definite relationship between both the Snyder test and the lactobacillus count and dental caries experience as measured by DMF teeth, there are many cases which cannot be easily categorized. This difference may be due to one of several factors. Both tests, as described in this paper, were correlated with cumulative caries experience rather than present caries activity. It is possible that had an index of caries activity been used a closer correlation of results might have been obtained. That would not fully explain, however, how some of those subjects with no clinical evidence of past or present caries activity will still show a positive bacteriologic result. This fact might be explained, as suggested by Jay[3] on a "healthy carrier" basis, that is, that while the laboratory picture tends to indicate some disorder, there is a clinical absence of the "disease" due to individual resistance or to some other factors.

Different salivary samples (3,147) were used as a basis of comparing the Snyder test results to the lactobacillus counts. The results of these comparisons are shown in Tables VII, VIII, and IX.

TABLE VII

RELATIONSHIP OF SNYDER TEST TO LACTOBACILLUS COUNT USING ROGOSA MEDIUM
(3,147 SAMPLES)

SNYDER TEST CLASSIFICATION	TOTAL NO. OF SAMPLES: 3,147	LACTOBACILLUS COUNT CLASSIFICATION				
		0 (708)	+1 (220)	+2 (440)	+3 (845)	+4 (934)
0	(675)	531	47	39	36	22
+1	(220)	74	34	40	40	32
+2	(370)	59	63	94	95	59
+3	(996)	27	64	211	370	324
+4	(886)	17	12	56	304	497

Utilizing the classifications as outlined in Table I, the highest relationships between the 2 tests appear at either extreme, that is, in the 0 and +4 categories. The results in the intermediary groups, while they do not correspond closely percentage-wise, do follow the same distribution pattern. This might indicate that the test classifications are not sufficiently definitive in this area but that the same factors influencing one of the tests also influence the

TABLE VIII

SNYDER COLORIMETRIC TEST AND LACTOBACILLUS COUNT RESULTS
(3,147 SAMPLES)

CLASSIFICATION	NO. OF SNYDER TESTS	NO. OF LACTOBACILLUS COUNT	NO. OF BOTH TESTS HAVING SAME RESULT	% OF SNYDER TESTS HAVING THE SAME LACTOBACILLUS COUNT	% OF LACTOBACILLUS COUNT HAVING THE SAME SNYDER TEST
0	675	708	531	78.7	75.0
+1	220	220	34	15.5	15.5
+2	370	440	94	25.4	21.4
+3	996	845	370	37.1	43.8
+4	886	934	497	56.1	53.2

TABLE IX

SNYDER COLORIMETRIC TEST AND LACTOBACILLUS COUNT RESULTS
(3,147 SAMPLES)

	SNYDER TEST	
CLASSIFICATION	% ALSO HAVING NEGATIVE LACTOBACILLUS COUNT	% ALSO HAVING POSITIVE LACTOBACILLUS COUNT
Negative (0)	78.7	21.3
Positive (+1, +2, +3, +4)	7.2	92.8
	LACTOBACILLUS COUNT	
CLASSIFICATION	% ALSO HAVING NEGATIVE SNYDER TEST	% ALSO HAVING POSITIVE SNYDER TEST
Negative (0)	75.0	25.0
Positive (+1, +2, +3, +4)	5.9	94.1

other to approximately the same degree. It will also be noticed, as shown in Table IX, that when the tests are grouped according to negative and positive results at least 75 per cent of the negative results and over 92 per cent of the positive results show a positive correlation. It is assumed that the lack of a perfect correlation is due to several factors. These include first, the ability of oral organisms other than lactobacilli to produce a low acidity[9, 10] on a glucose medium; second, to variance in fermentive ability[11] such that many homofermentive organisms may produce only a small change in pH while a few heterofermentive organisms may produce a large change in pH (only those Snyder tests which showed a complete color change in 96 hours were classified as having positive reactions); and third, to the possibility of the introduction of experimental error.

One thousand and sixteen subjects, or 3,048 salivary samples, were used to determine the reproducibility of the tests for 3 separate collections. These findings are summarized in Table X.

TABLE X

REPRODUCIBILITY OF TEST RESULTS FOR 3 COLLECTIONS
(1,016 CASES)

	3 OUT OF 3	2 OUT OF 3
Snyder test	38.4% (390)	85.6% (870)
Lactobacillus count	42.6% (433)	87.6% (890)

On the basis of these figures it would seem that if the median value of each test result for all 3 collections was used, an accurate reproducible value would be obtained in over 85 per cent of the cases.

CONCLUSIONS

1. There appears to be a high correlation between the Snyder colorimetric test and caries experience as measured by DMF teeth.

2. There appears to be a high correlation between the lactobacillus count using Rogosa medium and caries experience as measured by DMF teeth.

3. There appears to be a close parallel between the results obtained by the Snyder test and the lactobacillus count using Rogosa medium.

4. There appears to be a high degree of reproducibility of both the Snyder test and the lactobacillus count using Rogosa medium when based upon obtaining the same test results on 2 out of 3 different salivary samples from the same patient.

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THE RELATIONSHIP BETWEEN FLUORINE AND THE ACTIVITY OF THE THYROID GLAND ON THE INCIDENCE OF DENTAL CARIES IN THE RAT

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IN THREE previous studies, the activity of the thyroid gland has been shown to be intimately related to the incidence of dental caries in the rat.[1-4] The addition of various inorganic fluorides to experimental diets in the rat is known to reduce dental caries,[5-7] and since conflicting data[1, 3] have previously appeared regarding the ability of fluorine to reduce dental caries in animals with a decreased thyroid gland activity, it was considered important to re-evaluate this problem.

EXPERIMENTAL

A total of 310 weanling male albino rats (Sprague-Dawley strain) were equally divided according to initial body weight into 9 different experimental groups. Groups 1, 2, and 3 received 500 μc of I^{131} in a single dose administered subcutaneously. In addition, Group 1 received 0.1 per cent propylthiouracil in the diet, and Group 2 received sodium fluoride in the drinking water at a concentration of 25 μg F per milliliter. Groups 4 and 5 received desiccated thyroid in the diet (in increasing concentration from 10 to 20 mg. per day, depending upon the age of the animal). Group 5 also received fluorine in the drinking water at the same concentration as the animals in Group 2. Groups 6 and 7 received propylthiouracil in the diet (0.1 per cent), while Group 7 received, in addition, the same level of fluorine in the drinking water as Groups 2 and 5. The animals in Group 8 received fluorine at the same level as the animals in Groups 2, 5, and 7, while Group 9 received no supplement in either the food or drinking water and served as a control group.

All the animals were housed in pairs in raised screen cages in an air conditioned room. They received a stock corn cariogenic diet* and either fluorine-low distilled water or the fluoride supplements ad libitum. After the termination of the experimental period of 140 days, the animals were sacrificed by ether and the heads examined for dental caries by methods previously reported.[7]

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*The composition of the diet used in this study was as follows: Yellow corn grits, 52.7 per cent; ground yellow corn, 11.3 per cent; powdered whole milk, 30.0 per cent; alfalfa, 4.8 per cent; iodized sodium chloride, 1.0 per cent; and irradiated yeast 0.2 per cent.

DATA AND DISCUSSION

The anticariogenic effectiveness of desiccated thyroid and the cariogenic effect of thiouracil and I^{131} are again demonstrated in these experiments. The data in Table I indicate that the subcutaneous administration of radioactive iodine increases the incidence of dental caries by 32 per cent and the dietary administration of thiouracil increases caries by 29 per cent (significant: $p = 0.02$). The cariogenic effect of these 2 substances at these concentrations appear approximately equal. The anticariogenic effectiveness of fluorine is evident in the animals receiving I^{131} or thiouracil plus sodium fluoride in the drinking water by having less dental caries than when thiouracil or I^{131} were administered alone. Although the reduction in the fluoride-treated animals which also received thiouracil or I^{131} was not statistically significant in comparison to the groups receiving thiouracil or I^{131} alone, the tendency toward less caries is clearly evidenced by a reduction in the mean number of lesions and in fewer moler teeth affected. It remains to be determined if less caries in these groups is a result of the topical effect of fluorine or a synergism between the thyroid hormone with fluorine. However, experiments in which desiccated thyroid was administered to rats who also received systemic injections of fluoride suggests that the effect of fluorine is one of a topical nature.[8]

TABLE I
THE EFFECT OF I^{131}, THIOURACIL, DESICCATED THYROID, AND FLUORINE ON THE INCIDENCE OF DENTAL CARIES IN THE RAT

SUPPLEMENT	NUMBER OF RATS	MEAN NUMBER OF LESIONS	PER CENT DIFFERENCE	MOLARS AFFECTED	ANIMALS WITH DENTAL CARIES (PER CENT)
I^{131} + thiouracil	23	12.6 ± 1.9†	+42	6.3	100
I^{131} + F*	25	9.5 ± 2.4	+23	4.4	100
I^{131}	27	10.8 ± 2.2	+32	5.3	100
Desiccated thyroid	21	4.6 ± 1.8	−37	2.6	52
Desiccated thyroid + F	11	5.6 ± 2.2	−23	3.6	91
Thiouracil	24	10.3 ± 2.4	+29	5.0	100
Thiouracil + F	31	8.6 ± 1.9	+15	4.2	100
Control + F	24	6.2 ± 1.8	−15	3.6	100
Control	26	7.3 ± 2.0	--	3.6	100

*Fluoride as sodium fluoride.
†Standard deviation.

When the same animals received both I^{131} and thiouracil, a greater increase in dental caries was found than when either of these substances was administered alone. The increase in caries in the animals receiving both I^{131} and thiouracil was significantly greater when compared to the controls ($p = 0.001$) as well as when compared to the animals receiving I^{131} or thiouracil alone ($p = 0.04$). Whether this increased cariogenic effect is an indication of different effects of the 2 substances on the salivary glands or if it indicates simply a synergistic effect from the addition of 2 cariogenic substances is not understood.

It is yet not known whether an increase in flow per se or a modification in the saliva itself is responsible for the reduction in dental caries. If I^{131} and

thiouracil act by different mechanisms to increase dental caries, it must be determined if this is due to a modification of the saliva itself or to a decrease in salivary flow.

The reduction in dental caries afforded by feeding desiccated thyroid is highly significant ($p = 0.001$) and confirms previous work.[1-3, 8] However, when fluoride and desiccated thyroid were fed together the anticariogenic synergism previously observed[1] was not confirmed. This may not be a valid comparison, however, since in the previous experiment, much higher levels of thyroid were used and smaller numbers of animals remained at the termination of this study. The mortality of the animals seems to be markedly increased when both fluorine and desiccated thyroid are administered together. The anticariogenic effect of desiccated thyroid is superior to the effect produced in these experiments by sodium fluoride alone. While the group receiving sodium fluoride alone did not exhibit a significant reduction in the incidence of dental caries ($p = 0.35$), the tendency for anticariogenic effect is certainly indicated.

Microscopic examination of the salivary glands confirm previous observations on similarly treated animals. The most marked alterations occurred in the granular tubules of the submaxillary glands. Animals treated with I^{131} exhibited a dramatic diminution in size of these tubules, although the simultaneous administration of thiouracil or fluorine to I^{131} treated animals induced no further demonstrable changes in the salivary gland. The changes which occurred were indistinguishable from those seen in animals receiving thiouracil alone or thiouracil in combination with fluorine. Animals fed desiccated thyroid exhibited marked enlargement of the granular tubules. The simultaneous administration of fluoride to animals receiving desiccated thyroid induced no further changes. The salivary glands of animals receiving fluoride did not differ morphologically from control animals receiving no supplements.

The thyroid glands of animals receiving 500 μc I^{131} were extremely small but presented recognizable follicles in a few instances. Such persistent follicles were lined by extremely flattened epithelial cells and contained no colloid material. Fibrosis of the glands was a prominent finding and appeared to have replaced much of the thyroid tissue so that only occasional thyroid cells could be found. The thyroid glands from animals which received 500 μc I^{131} and in addition were supplemented by either thiouracil or fluoride were indistinguishable from animals receiving only I^{131}. When desiccated thyroid was administered alone or simultaneously with fluoride the follicles were large, contained a very homogeneous, intensely eosinophilic colloid, and were lined by extremely flattened epithelial cells. No differences could be noted between these 2 groups. In contrast, the control animals and animals receiving only fluoride exhibited thyroid glands which contained numerous swollen follicles. The follicles contained a vacuolated colloid which stained only lightly eosinophilic and which were lined by cuboidal to columnar epithelial

cells. Animals receiving thiouracil exhibited the characteristic hyperplastic, cellular thyroid gland which was indistinguishable from animals concomitantly receiving fluoride.

CONCLUSIONS

The cariogenic effects of thiouracil and I^{131}, and the anticariogenic effect of desiccated thyroid are again confirmed in this study. When thiouracil and I^{131} are administered together in the same animals, a significantly greater cariogenic effect results than if either are administered alone. The effect of providing fluoride in the drinking water of the animals receiving thiouracil or I^{131} resulted in less dental caries than if the animals received no added fluoride, thus suggesting an anticariogenic topical effect of the fluoride. No synergism was found between the effect produced by feeding desiccated thyroid and fluoride together.

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THE METABOLISM OF DIFFERENT FLUORIDES IN THE RAT

I. Comparisons Between Sodium Fluoride, Sodium Silicofluoride, and Stannous Fluoride

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THE use of fluoride compounds other than the sodium salt in fluoridation programs has been questioned,[1] since data are not available to indicate if all inorganic fluoride compounds furnish equal numbers of fluoride ions to the organism, thus commensurately reducing dental caries. McClure[2] concluded that sodium fluoride and sodium silicofluoride produce equal fluoride storage in the skeleton of the rat, thus indicating that equal amounts are metabolically available. However, no data are available in which the metabolism (the amount excreted in the feces and urine in relation to the amount ingested) of the 2 fluorides is compared in addition to the skeletal storage. The purpose of this paper is to investigate the metabolism of small concentrations of fluorine (at levels similar to those which are produced in artificially fortified fluorine-deficient communal water supplies) furnished as sodium fluoride, sodium silicofluoride, and stannous fluoride.[3-5]

PROCEDURE

A total of 9 rats (Sprague-Dawley strain) from three 30-day-old litters were divided into 3 experimental groups, so that each contained 1 animal from each litter. Distributions were made so as to maintain equal weight among groups. The animals had previously received a stock corn diet relatively low in fluorine (F = 0.5 μg per gram) and fluorine-free water. At the initiation of the experiment, 6 littermates of the animals used in this study were sacrificed and analyzed for fluorine.

Each experimental group received either sodium fluoride, sodium silicofluoride, or stannous fluoride added to the drinking water at a level of 1.0 μg fluorine per millimeter. In all instances, the same amount of fluoridated water was given to each animal during the experiment. The water was placed in a graduated cylinder on the outside of the cage. This was attached to a drinking tube which entered the cage through a small hole. A thistle tube placed directly beneath the drinking tube collected any water spilled during drinking and led it into a covered beaker. This spilled water was returned to the drinking tube and consumed before the termination of the daily period. If

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any animal consumed his allotted amount of fluoridized water before the termination of each daily period, redistilled fluorine-free water was offered ad libitum.

The diet was available ad libitum, but the amount consumed was measured so as to accurately estimate the amount of dietary fluorine ingested. The animals were housed individually in round, all stainless steel cages having a No. 2 mesh floor. The food cup, arranged to minimize spillage, was securely attached to the bottom of a 5-inch stainless steel side tube. The diet was weighed into the food cup and reweighed at the end of each 3-day period.

A thick filter paper to catch the feces and absorb the urine was placed in a Pyrex dish beneath each cage. The feces of each rat were collected and analyzed for fluorine at the end of each 3-day period by placing the entire sample in a small silica crucible to which was added 1 Gm. of low-fluorine calcium oxide. The feces and calcium oxide were mixed with a small amount of fluorine-free water and dried under an infrared lamp after which they were ashed at 550° to 600° C. for 6 hours in an electric muffle. The urine-containing filter papers were prepared similarly in silica evaporating dishes of suitable size. The fluoride was distilled by the procedure of Flagg[6] and the distillate was titrated by a procedure similar to that of Smith and Gardner.[7]

At the termination of the 40-day period, the animals were sacrificed and prepared for fluorine analysis by removing the skin to prevent external contamination, charring the entire carcass in a silica evaporating dish and ashing as previously described.[8] The total ash was weighed, pulverized with a glass rod, and transferred to a clean bottle. Multiple samples of the ash of each animal were analyzed, thus allowing the determination of the total amount of fluorine in the animal.

DATA AND DISCUSSION

The 40-day metabolism of the animals receiving each of the fluorides used in this experiment is shown in Table I. There does not appear to be any

TABLE I
TOTAL FLUORINE METABOLISM

SUPPLEMENT	TOTAL INGESTED (MG. F PER RAT)	TOTAL EXCRETED	
		URINE (MG. F PER RAT)	FECES (MG. F PER RAT)
Sodium fluoride	1.26	0.59	0.13
Sodium silicofluoride	1.28	0.55	0.14
Stannous fluoride	1.27	0.48	0.12

marked difference in the amount of fluorine in the feces of any of the various groups nor in the urinary fluorine level of the silicofluoride and the sodium fluoride group. However, the urinary level for the stannous fluoride group is somewhat less. On the basis of these data, an attempt was made to calculate the amount of fluorine which would be found in the ash of the animals of the various groups, by subtracting the total amount of fluorine excreted from the total fluorine ingested. To this difference was added the amount of fluorine found in the animals initially, estimated by analysis of the 6 littermates of the animals which were sacrificed at the beginning of the experiment.

The calculated fluorine retention in the skeleton is compared in Table II with that actually found by analysis of the ash of the animals at the termination of the experimental period. There is no apparent difference in the amount of fluorine found by analysis in either of the 3 groups, and the amounts found in the whole carcass agree quite well with the amounts calculated to be present.

TABLE II
MEAN FLUORINE RETENTION

SUPPLEMENT	CALCULATED (MG. F PER RAT)	FOUND (MG. F PER RAT)
Sodium fluoride	1.79	1.77
Sodium silicofluoride	1.83	1.79
Stannous fluoride	1.80	1.70

Previous data,[8, 9] obtained by determining fluorine storage alone, have suggested that various inorganic ions interfere with the metabolism of fluorine in the rat. Since neither urinary nor fecal values were determined in these previous studies, it was considered of interest to determine the effect on fluorine retention, as evaluated by metabolism studies, of administering the fluoride in the presence of calcium, since calcium is known to interfere with the utilization of fluorine. Such a study would serve to evaluate the accuracy of these experimental procedures.

A solution (A) containing approximately 1.0 mg. F/ml. and 1 per cent Ca^{++} was prepared by mixing 100 ml. of a solution containing 7.337 Gm. $CaCl_2 \cdot 2H_2O$ with 100 ml. of a solution containing 0.620 Gm. C.P. sodium fluoride. By a similar procedure, substituting redistilled water for the calcium chloride solution, a solution (B) containing 1.0 mg. F/ml. without added calcium was prepared for comparative purposes. Exactly 1.0 ml. of each solution was transferred by a volumetric pipette to a 100 ml. volumetric flask and following proper dilution, quadruplicate 4.0 ml. aliquots were transferred to the fluorine stills for analysis. Analysis indicated that solution A contained 1,120 ± 14 μg F/ml., and solution B contained 1,091 ± 15 μg F/ml. Therefore, 24 ml. redistilled water were added to solution A and 18 ml. redistilled water were added to solution B. Analysis (in quadruplicate) of these "corrected" solutions indicated 1,002 ± 8 μg F/ml. in solution A and 1,014 ± 10 μg F/ml. in solution B.

A litter of 7 rats was divided into 2 groups of 3 animals each; the remaining animal was sacrificed for carcass analysis to determine the approximate initial amount of fluorine in the carcass. A stock corn diet containing about 1 μg F/ml. was available to each animal ad libitum. The amount consumed was not measured, since this amount would be insignificant when compared to the rather large amount of fluorine to be administered. Groups A and B received 0.5 ml. of their respective solutions per day, administered by stomach tube.

Feces and urine were collected daily and prepared for fluorine analysis as previously described. The duration of the experiment was 14 days, after

which the animals were sacrificed and analyzed for fluorine as described previously. The total amount of fluorine administered (excluding that amount ingested with the diet) was 7.0 mg., and the amount of fluorine in the littermate animal at the beginning of the experiment was 52 μg.

The results of this experiment are summarized in Table III. Those rats which received the aqueous sodium fluoride solution without calcium excreted 1.876 mg. of fluorine in the urine, which was 26.6 per cent of the ingested fluorine. When the fluoride was ingested in the presence of calcium chloride 0.453 mg. of fluorine was excreted in the urine. These data corroborate previous work which indicated decreased storage of fluorine in the skeleton when administered in the presence of calcium chloride.[8] The fecal analyses similarly suggest that the calcium chloride has a pronounced effect on the absorption of fluorine, since the animals receiving the fluoride solution without calcium had only 10.5 per cent of the administered fluoride in the feces while the group receiving the fluoride solution with calcium chloride had 75.2 per cent of the fluorine in the feces. Similar conclusions are evident by examining the fluorine retained in the whole carcass, since 75.2 per cent of the ingested fluorine was found in the group which received the sodium fluoride solution and only 21.4 per cent was found when the same amount of fluorine was given in the presence of calcium chloride.

TABLE III
FLUORINE METABOLISM IN RATS RECEIVING FLUORINE IN A CALCIUM SOLUTION (A) AND IN DISTILLED WATER (B)

	FLUORINE EXCRETED						FLUORINE RETAINED	
	URINE		FECES		TOTAL			
GROUP	MG.	%	MG.	%	MG.	%	MG.	%
A	0.453	6.4	5.303	75.2	5.756	81.6	1.51	21.4
B	1.876	26.6	0.738	10.5	2.614	37.1	5.30	75.2

It is of some interest to note that the ratio of fluorine retained in the carcasses of the Group B animals compared to the Group A animals is 3.51, indicating that the absence of this amount of calcium resulted in 3.5 times as much fluorine storage. When the fluorine retention calculated from the total excretion data is compared, the ratio is 3.49, thus indicating the same relative results from the metabolism data as from the analysis of the carcasses. It may be seen from the feces data that the Group B animals apparently absorbed about 3.6 times as much fluorine as the Group A animals. This would seem to indicate that the retention of fluorine is almost directly proportional to the absorption. In this case, the difference in absorption was probably due to the insolubility of the calcium fluoride formed in solution A.

CONCLUSIONS

A method of measuring the metabolism of microconcentrations of fluorine in the rat has been utilized to determine the amount of fluorine found in the

feces and urine, and the whole carcass. Experimental data have been presented which indicate that good recovery of all of the fluorine given to the animals is possible.

When sodium fluoride, sodium silicofluoride, and stannous fluoride are administered in the drinking water at a fluorine concentration of 1.0 μg per milliliter, there are essentially no differences in the amount of fluorine found either in the feces, urine, or whole animal.

Calcium chloride has been shown to have a pronounced inhibiting action on fluorine absorption and retention within the skeleton. This has been studied by estimating the amount of fluorine in the feces, urine, and whole animal when identical amounts of fluorine were given by stomach tube to two groups of rats, one of which received the fluoride in aqueous solution and the other in a calcium chloride solution.

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THE EFFECT OF FLUORIDE INGESTION ON THE COMPOSITION AND SOLUBILITY OF MINERALIZED TISSUES OF THE RAT

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THE formation of fluorapatite in aqueous solution has been noted by MacIntire, Hardin, Oldham, and Hammond[1] in studies of the cause of phosphate insolubility, by Montel and Chaudron,[2] Rathje,[3] in their studies on the synthesis of various apatites, and McCann[4] in a study of the reactions of fluoride with hydroxyapatite. Neuman, Neuman, Main, O'Leary, and Smith[5] have shown that fluorapatite is formed below the solubility product of calcium fluoride by ionic exchange. McCann and Bullock[6] indicated that a number of reactions occur between fluoride ion and powdered enamel and dentin, but the principal one, up to a concentration of about 100 ppm F^-, is the formation of fluorapatite. Gerould,[7] Syrrist,[8] and Rathje[9] have presented evidence showing that it is quite probable that the principal reaction occurring between fluoride and the hard tissues of the body in vivo is the formation of fluorapatite.

It was the purpose of this study to obtain additional evidence of the formation of fluorapatite in vivo in skeletal and dental tissues. Information was obtained also on the properties of tissues fluorosed in vivo as well as in vitro, as shown by solubility and ion-exchange studies. A chemical analysis of control and fluorosed tissues furnished additional data on the effect of fluoride on tissue composition.

EXPERIMENTAL

Litters totaling 235 weanling Sprague-Dawley rats, weighing between 36 and 47 grams, were divided between 2 groups, each group receiving half of each litter. Both groups were fed an adequate diet low in fluoride (1 ppm) composed of cornstarch, 66.5 per cent; whole milk powder, 27.0 per cent; yeast, 5.0 per cent; cod liver oil, 1.0 per cent; salt mixture (500 Gm. NaCl, 53.2 Gm. ferric citrate trihydrate, and 3.9 Gm. copper sulfate pentahydrate), 0.5 per cent. One group received 100 ppm fluoride as NaF in the drinking water; the other group served as controls and received distilled water. The average body weight after 100 days on experiment was 176.2 Gm. for the 118 controls and 157.8 Gm. for the 117 fluoride-treated rats. After sacrifice the animals were autoclaved and the femora, mandibles, humeri, molars, and incisors removed and pooled by groups. Fat was extracted with alcohol and ether, and the samples dried, powdered to pass a 60-mesh sieve, and the enamel and dentin of the molars and incisors separated by the method of Manly and Hodge.[10]

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Analytical Methods.—Samples were analyzed in duplicate or triplicate for calcium, phosphorus, magnesium, fluoride, sodium, and carbon dioxide as described previously[6] with a precision of 0.15, 0.2, 4, 3, 2, and 1 per cent respectively.* Some of the solubility tests described below were made by titrating the combined calcium and magnesium present in the filtered solutions with the sodium salt of ethylenediamine tetraacetic acid using Erichrome Black T as indicator.[11]

Solubility Studies.—The solubility of each tissue was determined by adding 0.250 Gm. of solid to 1 liter of water in a polyethylene bottle and shaking occasionally for a period of 30 days. The temperature was maintained at 37° C. by storage in a constant temperature room. The filtrate was then analyzed for calcium and phosphorus. Attempts to determine the magnesium and fluorine were unsuccessful due to the low concentration of these components. The comparative solubilities of several of the samples in lactic, acetic, and hydrochloric acids were obtained by shaking 0.050 Gm. of solid in 25 ml. of solution for 48 hours in a mechanical shaker at room temperature (about 30° C.). Solubility was then determined by titrating the combined magnesium and calcium as described above.

Rate of Fluoride Uptake in Vitro.—The rate of the in vitro reaction between fluoride ions and control tissue was determined at 37° C. by adding 0.1 Gm. of the solid to 800 ml. of solution containing the desired concentration of fluoride as NaF. The samples were stirred constantly for a specified length of time, then filtered through sintered glass, and the solid analyzed for fluorine.

Exchange Studies With KOH.—One to 2 tenths gram samples of this solid were added to 50 ml. of 1 N KOH maintained at 37° C. for a period of 1 to 7 days with occasional shaking. Samples were then filtered, the paper ignited, and both liquid and solid analyzed for fluorine.

RESULTS

Analysis of Tissues (Table I).—The amount of fluoride deposited is a function of the kind of tissue involved. The 3 different bones have about the same per cent of fluoride, the molar and incisor dentin have a lower content, and the molar and incisor enamel lower yet. The samples of molar and incisor enamel, in contrast to the bone and dentin samples, differ somewhat from each other, probably because of a difference in the way fluoride is deposited in enamel as compared with that in dentin and bone.

It is interesting to note that the bones from the fluoride-treated rats contain less CO_2 than the controls, whereas the CO_2 content of the molar dentin remains practically constant. The controls vary from 2.57 to 3.01 per cent; tissues from the fluoride-treated rats range only from 2.61 to 2.66, which is constant within the absolute experimental error of ± 0.03 per cent. However, the CO_2 content of the incisor dentin of both control and fluoride-treated rats is considerably different, which is true of its magnesium content as well (1.49 and 1.92 per cent vs. about 0.4 per cent for molar dentin).

*Calculated as coefficient of variation.[12]

TABLE I
ANALYTICAL DATA ON DRY, FAT-EXTRACTED RAT TISSUES

SAMPLE	Ca (%)	P (%)	Ca:P (RATIO)	F (%)	Mg (%)	Na (%)	CO_2 (%)	ASH (%)
Femora—Control	24.38	12.28	1.536	0.010	0.60	0.50	2.76	67.3
Femora—Fluoride	24.70	12.25	1.560	0.832	0.74	0.53	2.64	69.0
Humeri—Control	24.51	12.13	1.564	0.013	0.65	0.50	2.87	67.9
Humeri—Fluoride	25.23	12.48	1.565	0.850	0.74	0.55	2.66	70.5
Mandibles—Control	26.56	13.28	1.547	0.012	0.64	0.53	3.01	73.6
Mandibles—Fluoride	26.28	12.96	1.568	0.822	0.72	0.63	2.63	73.5
Molar dentin—Control	28.03	14.04	1.545	0.009	0.37	0.58	2.57	77.2
Molar dentin—Fluoride	28.56	14.10	1.567	0.480	0.44	0.70	2.61	78.2
Incisor dentin—Control	26.80	15.41	1.345	0.004	1.49	---	1.92	79.5
Incisor dentin—Fluoride	27.56	15.26	1.397	0.521	1.92	0.76	1.97	80.8
Molar enamel—Control	35.32	17.43	1.568	0.004	0.32	0.73	---	---
Molar enamel—Fluoride	34.86	17.35	1.555	0.077	0.37	0.79	---	---
Incisor enamel—Control	34.74	17.35	1.550	0.002	0.25	0.66	---	---
Incisor enamel—Fluoride	33.26	16.90	1.522	0.197	0.66	0.81	---	---

In every case the magnesium content is higher in the fluoride-treated animals than in the controls. Particularly striking is the increase in magnesium content of the incisors of the rats which received fluoride, especially since the incisors were completely renewed during the experiment, and all the magnesium found on analysis (present at sacrifice) had been incorporated during the test period.

Composition of Tissues on the Ash Basis (Table II).—In each of the bones the incorporation of fluoride has not increased the calcium content of the ash. There is in fact a slight decrease in calcium, although the calcium to phosphorus

TABLE II
COMPOSITION OF TISSUES ON ASH BASIS

SAMPLE	Ca (%)	P (%)	F (%)	Mg (%)	CO_2 (%)
Femora—Control	36.22	18.25	0.02	0.89	4.10
Femora—Fluoride	35.80	17.76	1.21	1.07	3.83
Humeri—Control	36.10	17.86	0.02	0.95	4.23
Humeri—Fluoride	35.78	17.69	1.21	1.05	3.77
Mandibles—Control	36.09	18.04	0.02	0.87	4.09
Mandibles—Fluoride	35.75	17.63	1.12	0.98	3.58
Molar dentin—Control	36.32	18.18	0.01	0.48	3.33
Molar dentin—Fluoride	36.52	18.03	0.61	0.56	3.34
Incisor dentin—Control	33.71	16.39	0.05	1.88	2.42
Incisor dentin—Fluoride	34.12	18.89	0.64	2.38	2.42

ratio increased slightly as a result of a somewhat larger reduction in phosphorus content. In the dentin there is a slight increase in the per cent of calcium. However, the effect on the ash of the bone appears to be negligible. The magnesium in the ash of all tissue was increased by fluoride.

Solubility Tests (Tables III and IV).—A considerable reduction in water solubility after in vivo fluoride exposure occurred with most of these tissues as seen in Table III. The reason for the difference between calcium versus phosphorus solubility in the molar dentin compared to that of the other tissues is not known. The difference in the Ca:P ratio of the solution of incisor dentin as compared with the other tissues may be accounted for by the difference in

TABLE III

SOLUBILITY OF TISSUES IN WATER

SAMPLE	Ca (MG./1)	P (MG./1)	Ca:P (RATIO)
Femora—Control	13.60	7.55	1.394
Femora—Fluoride	9.08	5.50	1.276
Humeri—Control	13.65	7.64	1.382
Humeri—Fluoride	8.38	5.50	1.178
Mandibles—Control	13.60	6.45	1.629
Mandibles—Fluoride	10.55	5.14	1.587
Molar dentin—Control	20.7	7.05	1.227
Molar dentin—Fluoride	10.11	5.72	1.367
Incisor dentin—Control	9.55	9.33	0.792
Incisor dentin—Fluoride	7.08	6.85	0.800
Molar enamel—Control	5.90	3.00	1.521
Molar enamel—Fluoride	5.55	2.80	1.533
Incisor enamel—Control	7.00	4.50	1.202
Incisor enamel—Fluoride	4.20	2.35	1.382

the Ca:P ratio of the solid (Table I). In the case of molar enamel the decrease in solubility was slight, possibly on account of the small amount of fluoride incorporated. Further solubility studies were carried out using lactic, acetic, and hydrochloric acids of various strengths as seen in Table IV. In each case the solubility of the tissues from the fluoride-treated rats was considerably less than that of the corresponding controls. The solubility was then compared with samples of control tissues treated in vitro with fluoride ion at a concentration of 2 ppm for a period of 2 months at 37° C. with occasional shaking. This material had a fluorine content somewhat higher than the material obtained from the rats in vivo (femora, 1.13 per cent vs. 0.83 per cent; incisor dentin, 0.87 per cent vs. 0.52 per cent; molar dentin, 0.85 per cent vs. 0.48 per cent), and was assumed to contain most of its fluorine as fluorapatite as shown by previous work.[6] These tissues gave solubilities in several acidic solvents close to the in vivo-treated material in the case of the femora and incisor dentin. The molar dentin treated controls were somewhat less soluble than the in vivo fluoride samples (Table IV).

TABLE IV

COMPARATIVE SOLUBILITIES*

SAMPLE	WATER	LACTIC ACID			ACETIC ACID			HYDROCHLORIC ACID		
		.00001 N	.0001 N	.001 N	.00001 N	.0001 N	.001 N	.00001 N	.0001 N	.001 N
Femora										
Control	18.2	22.2	21.9	29.1	22.9	18.2	21.2	22.5	26.2	58.3
Fluoride	13.9	13.9	14.2	15.9	14.2	15.9	16.9	15.9	15.9	45.0
F-treated control	13.3	14.2	14.2	16.6	14.2	14.6	15.9	13.6	15.3	42.2
Incisor Dentin										
Control	28.8	28.8	30.8	39.4	26.8	25.8	37.4	26.5	30.1	67.2
Fluoride	13.6	14.2	18.2	25.1	13.6	17.5	25.2	13.9	19.9	47.6
F-treated control	13.9	11.1	13.0	26.8	13.9	14.8	25.0	16.7	18.5	59.2
Molar Dentin										
Control	25.0	30.0	30.9	40.9	25.0	30.9	38.9	30.5	32.6	71.8
Fluoride	14.8	17.6	24.1	31.5	17.6	17.6	31.8	18.5	29.7	59.2
F-treated control	11.1	13.9	14.8	23.1	13.0	14.8	23.1	15.7	20.4	59.2

*In milligrams Ca + Mg per liter.

Another type of in vitro study was then carried out on the in vivo fluoride-treated tissues. It was thought that if the fluoride were present in the tissues as fluorapatite it would exchange with hydroxyl ion (as supplied in 1 N KOH). However, a test of these tissues showed that very little of the fluoride could be exchanged (about 5 per cent in 3 hours, 15 to 18 per cent in 1 to 7 days). Megirian[13] failed to desorb any fluoride from glycol-ashed bone previously treated with NaF solution for 4 days. The hydroxyl ion concentration used by Megirian was much lower than that employed here, in addition to the fact that the tissue had been ashed. The same procedure (treatment with KOH) was also carried out on control material that had been treated for a short time with fluoride in vitro, and it was found that most of the fluorine could be exchanged. A more thorough study was then made, the results of which are shown in Fig. 1. Thus, for example, in the case of the femora it can be seen that

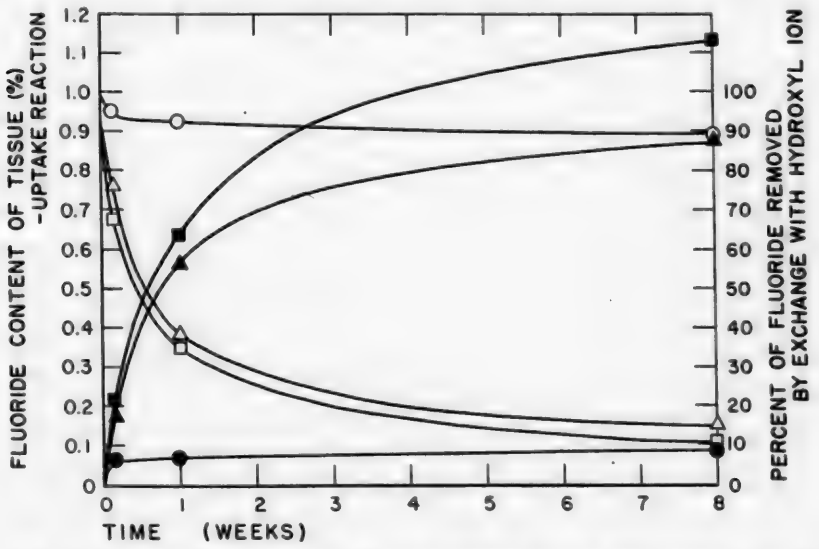

Fig. 1.—Rates of in vitro reaction of hard tissues showing fluoride uptake (■, ▲, ●), and amount of this fluoride exchanged by 1 N KOH solution (□, △, ○). The dentin curves represent both molar and incisor dentin which gave almost identical results. Femur is denoted ■, □; dentin ▲, △; and enamel ●, ○.

in 1 day's exposure to 2 ppm F^- at 37° C., the fluoride content is increased to 0.21 per cent. Of this, 68 per cent can be removed by 1 N KOH in 1 to 7 days. In 1 week the fluoride increases to 0.63 per cent of which 34 per cent can be removed by treatment with KOH.

The speed of reaching equilibrium in the reaction of fluoride ion with powdered bone (femur) and dentin is seen to be a relatively slow process, while the reaction of fluoride (ions) with enamel, although quantitatively limited, reaches equilibrium rapidly (Fig. 1). When an attempt is made to exchange the fluoride from in vitro-treated tissues with hydroxyl ions, the difference in the reaction of bone and dentin as compared with enamel is again

evident, that is, most of the fluoride can be exchanged from the enamel very quickly regardless of time of fluoride treatment; however, the fluoride can be removed from dentin and bone only if the material had been treated for a very brief time with fluoride ion. As the time of fluoride treatment increases the per cent that can be removed by KOH treatment decreases. Apparently the fluoride is only on the outer surfaces of the crystals on short exposure and penetrates deeper with time.

DISCUSSION

The analysis of hard tissues of rats receiving 100 ppm fluoride in drinking water as compared with tissues from control rats, has indicated that this amount of fluoride has little effect on the calcium and phosphorus content but does have an effect on the carbon dioxide and magnesium content. The results on CO_2 and magnesium are in agreement with the work of Kick et al.[14] rather than that of Wadhwani[15] which was in direct contrast to that of earlier workers. Previous work by Hendricks[16] and McCann and Bullock[6] has indicated that magnesium is present in calcified tissues, not as an integral part of the crystal, but bound on the surfaces by adsorption. Possibly the incorporation of fluoride in the tissues caused larger amounts of magnesium to be adsorbed because of the affinity of fluorine for magnesium. Apparently calcium, being an integral part of the crystal, is not affected in the same manner.

There is a notable similarity between the products of fluoride acting in vivo and the products formed by fluoride acting in vitro, i.e., by allowing the control tissues to react with 2 ppm fluoride for as long as 8 weeks. This latter reaction in vitro is known to form fluorapatite.[6] A similarity in behavior of the in vitro versus in vivo fluorosed tissues is exhibited in the solubility studies. Likewise, in the exchange studies with KOH, there is a difference in behavior shown by the samples treated in vitro with fluoride for a few hours or days versus a treatment for 8 weeks. The products of this longer treatment period in vitro become increasingly similar to the reaction products formed in vivo. Evidently with the increasing reaction time in vitro fluoride penetrates further into the crystals and its reaction becomes more and more similar to the reaction which occurs in vivo. This evidence seems to support the formation of fluorapatite in the tissues which were fluorosed in vivo.

Equilibrium rate studies with fluoride ions showed that for dentin and bone the attainment of equilibrium is a slow process. The most probable explanation for this is that the reaction is taking place on the surface by ionic exchange,[6] but in order to proceed further, recrystallization must take place probably by the process of thermal aging, as shown by Weikel and Neuman[17] and Neuman and Mulryan[18, 19] in the case of phosphate exchange in unashed bone. Since the enamel crystals are larger than those of dentin and bone,[20] recrystallization apparently takes place too slowly to be detected in a period of 2 months. The small amount of organic material as compared with dentin and bone may also be a factor. An apparent equilibrium therefore is observed in a few hours. Bone and dentin on the other hand appear to permit a relatively rapid recrystallization so that the reaction with fluoride continues

at a noticeable rate for 60 days and perhaps longer. The formation of fluorapatite apparently takes place by ionic exchange through a process of recrystallization into the crystal after surface positions are exchanged.

It has been pointed out by Hendricks,[16] and has been shown in previous work with powdered enamel and dentin,[6] that enamel has hidden surfaces that are not reached unless the crystals are ground extremely fine, or, of course, are actually dissolved by severe attack as with acid. Substances that are incorporated during formation are trapped on these surfaces and are not released except by an extremely slow reaction, when made accessible by some treatment. It is believed that bone and dentin can pick up considerable amounts of fluoride by adsorption into the tissues through ionic exchange and diffusion after the tissue has been completely formed, as well as by incorporation during growth. However, if enamel can pick up only a very limited amount of fluoride on its outer surface, the greater part of its fluoride content must be incorporated during growth.* This is indicated by the relative fluoride contents of the dentin and of the enamel in the molars versus the incisors.

SUMMARY

When rats are given fluoride at the level of 100 ppm in the drinking water under the conditions of this experiment, the calcium content of the bone is not increased with the increase in fluoride content. It was also shown that there is a tendency for the magnesium content of the tissues to increase with increase in fluoride content.

Additional evidence has been obtained to show that the material formed by the in vitro reaction of fluoride ions with dentin and bone may be fluorapatite probably produced by ionic exchange and recrystallization, and shows reactions similar to that produced by the in vivo reaction with fluoride (solubility and hydroxyl exchange). However, when enamel is treated in vitro the reaction presumably takes place mostly on the outer surfaces because of the inability of fluoride ion to penetrate to the hidden area of the crystal. This is probably because of the extremely slow thermal aging of enamel as opposed to that of bone and dentin. Fluoride, however, is incorporated throughout the tissue in the in vivo reaction.

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*The rat incisor tissue was completely renewed during the experiment, whereas the molars increased in weight only about 50 per cent and essentially all of this weight was due to the secondary cementum and dentin.

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EFFECT ON DENTAL CARIES OF A DENTIFRICE CONTAINING STANNOUS FLUORIDE AND DICALCIUM PHOSPHATE

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PREVIOUS clinical studies with a dentifrice containing stannous fluoride and heat-treated calcium phosphate have shown significant reductions in the dental caries experience of both children[1-3] and adults,[4] while a similar product containing sodium fluoride was ineffective in reducing the dental caries experience in children.[3] These studies, together with clinical topical studies[5-9] and many in vitro and animal tests, suggest that the particular fluoride compound used is of major importance with respect to the anticariogenic effectiveness of a dentifrice.

Besides the use of an effective fluoride compound, a second important consideration in the utility of an effective anticariogenic fluoride dentifrice is the nature of the polishing agent. Clinical tests had shown that a dentifrice containing sodium fluoride with the conventional polishing agent, dicalcium phosphate or calcium carbonate, was ineffective in reducing the dental caries rate.[10, 11, 12] Nevertheless, the apparently greater activity of stannous fluoride pointed to the possibility that this compound might be effective even in a formulation prepared with dicalcium phosphate. The present study was designed to investigate this possibility.

EXPERIMENTAL

The clinical procedure for conducting this study was identical with that reported previously.[1] Three groups of children, ranging in ages from 6 to 15 years, were used. Group 1 was composed of children who used a nonfluoride dentifrice with dicalcium phosphate as the polishing agent. Group 2 used an identical dentifrice except that it contained 0.4 per cent stannous fluoride (1,000 μg F/Gm. and 3,000 μg Sn/Gm.). Group 3 used a dentifrice with heat-treated calcium phosphate as the polishing agent and 0.2 per cent sodium fluoride (1,000 μg F/Gm.) plus sufficient stannous gluconate to make a final tin concentration of 3,000 μg Sn/Gm.

The compositions of the 3 dentifrices used throughout this study are shown in Table I. Each subject was supplied with the appropriate dentifrice in sufficient quantity to be used by himself and his entire family in order to eliminate the possibility of other dentifrices being used. The 3 dentifrices were indistinguishable by color or taste, and no attempt was made to suggest or demonstrate the frequency of use or method of toothbrushing.

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DATA AND DISCUSSION

The caries increments associated with the unsupervised use of the two fluoride-containing dentifrices and the control dentifrice after 6 months and 1 year are shown in Table II. This table indicates the per cent reduction in the dental caries rate in terms of the DMF tooth and DMF surface indexes. Following 6 months of use, the group using the stannous fluoride dentifrice in which the polishing agent was dicalcium phosphate showed 9.7 per cent more new DMF teeth, and 2.4 per cent fewer new DMF surfaces per subject than did the control group. After one year, the reduction in dental caries was 3.0 and 7.0 per cent, respectively, as indicated by the DMF tooth and DMF surface indexes. None of these differences are significant.

TABLE I
COMPOSITION OF THE EXPERIMENTAL DENTIFRICES USED BY CHILDREN, AGES 6 TO 15 YEARS, THROUGHOUT A ONE-YEAR TEST PERIOD

DENTIFRICE CONSTITUENT	GROUP		
	NO. 1 (%)	NO. 2 (%)	NO. 3 (%)
Dicalcium phosphate	47.0	47.0	—
Calcium pyrophosphate	—	—	40.0
Detergent (non-soap)	1.5	1.7	1.7
Humectant	24.0	24.0	30.0
Binder	1.6	1.6	1.5
Water	25.9	25.3	25.3
Stannous fluoride	—	0.4	—
Sodium fluoride	—	—	0.2
Stannous gluconate	—	—	1.3
Flavor	q.s.	q.s.	q.s.

The children in Group 3 who used a sodium fluoride-stannous gluconate dentifrice in which heat-treated calcium orthophosphate was the polishing agent showed approximately 13 per cent less dental caries by both indexes after 6 months' use. After 1 year there was 9.0 per cent less caries as indicated by the DMF teeth index and 15.0 per cent when the DMF surfaces index was used. None of these differences are significant.

These latter data are interesting in light of the previously published negative clinical data when sodium fluoride was used in a dentifrice containing heat-treated calcium orthophosphate,[3] dicalcium phosphate,[10] or calcium carbonate,[11, 12] all of which would suggest that sodium fluoride does not have a pronounced anticariogenic effect when any of the above polishing agents are used. The idea that the stannous ion, in combination with the fluoride ion, has a synergistic action is not dramatically evident in these data even though a fluoride-compatible abrasive was used. However, previous experience with combinations of sodium fluoride-stannous chloride mixtures fed to rats in their drinking water has suggested that the tin in such a combination of solutions is no more effective than comparable levels of fluorine in the absence of tin.[13] These animal data were suggestive of the idea that stannous fluoride itself resulted in the optimal conditions for demonstrating the anticariogenic effect of fluorine and the synergistic effect of the stannous ion. This is strengthened

by the fact that stannous fluoride has a pronounced anticariogenic effect in both children[1-3] and adults[4] when used with heat-treated calcium orthophosphate as the polishing agent.

TABLE II
DENTAL CARIES INCREMENTS DURING THE ONE-YEAR TEST PERIOD

	AFTER 6 MONTHS			AFTER 12 MONTHS		
	CONTROL	SnF_2	Sn GLUCONATE-NaF	CONTROL	SnF_2	Sn GLUCONATE-NaF
DMF teeth increment, average	1.40	1.56	1.22	2.24	2.18	2.03
Per cent reduction of control caries rate		–9.7	13.0		3.0	9.0
Probability		0.33	0.25		0.4	0.4
DMF surface increment, average	2.70	2.62	2.35	4.70	4.39	4.00
Per cent reduction of control caries rate		2.4	13.0		7.0	15.0
Probability		0.4	0.1		0.4	0.12
Number of subjects	215	223	221	178	210	198

SUMMARY

After 6 months and one year of unsupervised use of a dentifrice containing stannous fluoride and dicalcium phosphate as the polishing agent, no significant reductions in the dental caries rates were found. Similarly, when both sodium fluoride and stannous gluconate were added to a dentifrice containing heat-treated calcium orthophosphate, no significant reduction in dental caries was found. These data suggest that not only is the fluoride compound of importance in determining the anticariogenic effectiveness of fluoride dentifrices, but also the nature of the polishing agent deserves important consideration.

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EFFECT OF HORMONES ON STRUCTURE AND PROTEOLYTIC ACTIVITY OF SALIVARY GLANDS

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THE purpose of this study was to investigate the serial histologic and enzymatic changes occurring in the submaxillary glands of rats receiving either desiccated thyroid or thiouracil and, in addition, to determine whether desiccated thyroid and testosterone, separately or combined, prevent the regressive histologic and enzyme changes known to occur in the submaxillary glands following hypophysectomy.

A proteolytic enzyme with trypsinlike activity previously has been described in the submaxillary glands of both rats and mice.[1, 2] Sreebny[1, 3] characterized this enzyme in rats and suggested its site as being the serous or "granular" tubule portion of the gland. He found that the enzyme's concentration varies with the sex and age of the animal and that the enzymatic activity increases proportionally with age. Sreebny[4] also reported that the removal of the hypophysis caused a reversal of the puberal changes in this proteolytic activity. Junqueira[5] found that the protease content of submaxillary glands of castrated animals could be modified by the injection of sex hormones, testosterone being the most effective, while other studies by Junqueira, Fajer, Rabinovitch, and Frankenthal[2] with mice, which portray sexual dimorphism of the salivary glands, demonstrated comparable sexual variation in this enzymatic activity.

The histologic alterations in the rat submaxillary gland following hypophysectomy consist in part of a decrease in both the size of the serous tubules and their granular content.[4, 7, 8] Shafer and Muhler[8] have correlated the dental caries experience with the decreased proteolytic and amylolytic activity in the submaxillary glands of hypophysectomized rats, as well as with the histologic alteration of the salivary glands. They also studied the histologic changes occurring in the salivary glands following the administration of desiccated thyroid, testosterone, and propylthiouracil in rats.[9] Grad and Leblond[6] and Eartly and Leblond[7] showed a partial restoration in size and granular content of the serous tubules in young adult thyroidectomized rats treated with thyroxine, while testosterone plus thyroxine were fully effective in restoration of size and granular content after thyroidectomy. Neither testosterone nor thyroxine completely restored the submaxillary glands of hypophysectomized animals and it was suggested, therefore, that the serous granules appeared to

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be under direct control of an hypophyseal factor. However, Gabe[10] previously had reported that thyroxine did reverse the atrophy of the granular tubules in the gland of male hypophysectomized rats and Lacassagne and Chamorro[11] concluded in an experiment with mice that the marked changes in the salivary glands concomitant with hypophysectomy could be reversed by prolonged treatment with testosterone. These conflicting reports suggest that additional study is needed in this area.

MATERIAL AND METHODS

This study was divided into 2 series in order to evaluate the effect of the thyroid gland hormone on proteolytic activity and histologic structure of the salivary glands (Series I) and the effect of hypophysectomy with subsequent hormone replacement (Series II).

In Series I, 73 female Sprague-Dawley rats were divided according to initial body weight into 3 groups. Group 1 were controls, Group 2 received desiccated thyroid in the diet (10 to 20 mg. per day calculated according to body growth), and a third group received 0.1 per cent propylthiouracil in the diet. All animals were housed in raised screen cages and received a coarse corn diet[12] and distilled water ad libitum. In this series, 4 to 5 animals in each of the 3 groups were sacrificed at the following intervals; 3, 6, 9, 12, and 15 weeks. All animals were sacrificed by chloroform inhalation, and the salivary and thyroid glands were removed and fixed in Zenker-formol for histologic examination. The proteolytic activity was determined using the opposite combined submaxillary and sublingual glands by methods previously reported.[1, 8, 13, 14]

In Series II, 110 female weanling Sprague-Dawley rats were hypophysectomized* and 20 female unoperated animals were used as controls. The operated animals were divided according to body weight into 4 experimental groups. Group 1 received desiccated thyroid (between 5 and 10 mg. per day in the diet); Group 2 received testosterone† (aqueous suspension, injected intramuscularly, in increasing amounts from 2.5 to 10 mg. per week); Group 3 received desiccated thyroid in the same amounts as the animals in Group 1, plus weekly injections of testosterone at the same level as the animals in Group 2. Group 4 consisted of hypophysectomized animals receiving no diet supplement or injections. All animals were housed and fed as in the former series. In this series, 3 to 4 animals of each group were sacrificed at the following postoperative intervals; 1, 2, 4, and 6 weeks. The animals were sacrificed by chloroform inhalation and, as in the previous series, the salivary glands were divided for microscopic examination and analysis of proteolytic activity.

RESULTS

Histologic Studies: Histologic observations on the salivary glands of the animals in Series I have confirmed previous reports[9] concerning the effects

*Hormone Assay Laboratories, Chicago, Ill.

†The testosterone (Oreton-F) used in this study was generously supplied by Schering Corporation, Bloomfield, N. J.

of desiccated thyroid and propylthiouracil. Marked differences were noted between control animals and those receiving propylthiouracil or desiccated thyroid. After 6 weeks, the submaxillary glands of rats receiving desiccated thyroid resembled mature animals, with large, granule-choked tubules scattered in profuse numbers throughout the glands. Animals of this same period receiving propylthiouracil exhibited small submaxillary glands with few tubules and only sparse granules. By 9 weeks, animals receiving propylthiouracil still resembled immature animals with scarce tubules, few of which contained any granules. However, in the thyroid-treated group, there was a remarkable increase in the size of the gland tubules and the tubular cells contained dense numbers of granules. After 12 and 15 weeks, the alterations in the structure of the submaxillary glands were similar to those described above except that the changes were even more intense.

It was interesting that 3 animals receiving propylthiouracil for 15 weeks, exhibited a complete reversal of the histologic picture indicative of thiouracil-treated animals when placed on the diet containing desiccated thyroid. After receiving this diet for 4 weeks, the submaxillary glands resembled those of animals receiving desiccated thyroid for the 15-week period.

In the second portion of this study (Series II), all animals were sacrificed before maturity. Thus, the submaxillary glands had not achieved features by which they could be readily distinguished from untreated hypophysectomized animals. However, it was found that the administration of desiccated thyroid alone or desiccated thyroid and testosterone in combination to hypothyroid alone or desiccated thyroid and testosterone in combination to hypophysectomized animals did not result in the premature enlargement of submaxillary gland tubules and the appearance of granules noted previously in unoperated animals of a similar age receiving these compounds.

Proteolytic Enzyme Studies.—The control animals in Series I exhibited a progressive increase in both relative proteolytic activity and activity per gram of tissue concomitant with the growth of the animals (Table I). Animals receiving desiccated thyroid showed a striking increase in both relative activity and activity per gram of tissue over the control animals. In contrast, the propylthiouracil-treated animals manifested a low level of activity throughout the entire study. The thiouracil appeared to act by maintaining the gland in its immature state throughout the study. It is interesting that the 3 animals which received propylthiouracil for 15 weeks and subsequently received desiccated thyroid for 4 additional weeks exhibited the highest activity of any of the groups. This indicated that the suppression of proteolytic activity induced by thiouracil is a reversible phenomenon under these conditions.

The range of variation in activity between female animals of the same group was considerably higher than that experienced in males in this laboratory in other studies. This finding corroborates similar results obtained by Sreebny.[1]

TABLE I
THE EFFECT OF THYROID AND THIOURACIL ON THE PROTEOLYTIC ACTIVITY OF SUBMAXILLARY-SUBLINGUAL SALIVARY GLANDS OF RATS (SERIES I)

TIME PERIOD (WEEKS)	GROUP	NO. OF ANIMALS	AVG. WT. ANIMALS (GM.)	AVG. WT. SUBMAX. + SUBLING. GLAND (1 GLAND) (MG.)	PROTEOLYTIC ACTIVITY	
					RELATIVE ACTIVITY*	ACTIVITY PER GM.†
3	Control	5	113	121.2	1.16	2.00 ± 0.42‡
	Thyroid	5	110	138.0	2.44	3.53 ± 0.81
	Thiouracil	5	82	92.1	0.94	2.10 ± 0.43
6	Control	5	161	146.1	2.79	3.78 ± 1.31
	Thyroid	5	120	170.0	7.01	8.37 ± 1.16
	Thiouracil	5	91	97.7	1.40	2.92 ± 0.11
9	Control	5	177	162.0	4.31	5.47 ± 1.18
	Thyroid	5	120	176.5	7.88	9.36 ± 2.39
	Thiouracil	5	109	116.3	1.63	2.90 ± 0.51
12	Control	5	177	168.2	5.55	6.73 ± 1.53
	Thyroid	5	178	208.8	7.14	6.95 ± 2.01
	Thiouracil	5	130	133.1	2.84	4.51 ± 1.41
15	Control	3	216	198.3	4.99	5.15 ± 0.36
	Thyroid	3	196	209.8	7.95	7.85 ± 1.79
	Thiouracil	4	133	119.6	1.59	2.71 ± 0.43
19	Thiouracil (15 weeks) + Thyroid (4 weeks)	3	166	195.8	9.29	9.72 ± 0.15

*Expressed as the ratio between the color produced by the total gland and the color of the tyrosine standard.

†Expressed as the ratio between the color produced per gram of gland tissue and the activity of the tyrosine standard.

‡Standard deviation.

In Series II, little differences in proteolytic activity were noted between any of the groups during the 1- and 2-week period of the study (Table II). By the 4- and 6-week postoperative periods, the unoperated animals exhibited the progressive increase in activity noted in Series I. All other groups remained at a consistently low level of activity with the exception of the hypophysectomized group which received both testosterone and desiccated thyroid for 6 weeks. In this group, although the activity was somewhat higher than that of the other hypophysectomized groups, it still did not approach that of the control animals. The proteolytic activity of the salivary glands from hypophysectomized animals could not be re-established at a level comparable to control animals by any procedure utilized in this study.

DISCUSSION

The results of these studies add further support to previous observations that the salivary glands are controlled by both the pituitary and thyroid glands. Dysfunction of either organ results in alterations in both the structure and proteolytic enzyme activity of the salivary glands but, at present, the significance of these alterations is not clearly defined.

In the present studies, it has been possible to demonstrate that the increase in size and granular content of the serous tubules of the submaxillary

TABLE II
THE EFFECT OF THYROID AND TESTOSTERONE ON THE PROTEOLYTIC ACTIVITY OF SUBMAXILLARY-SUBLINGUAL SALIVARY GLANDS OF HYPOPHYSECTOMIZED RATS (SERIES II)

TIME PERIOD (WEEKS POST-OPERATIVE)	GROUP	NUMBER OF ANIMALS	AVG. WT. ANIMALS (GM.)	AVG. WT. SUBMAX. + SUBLING. GLAND (1 GLAND) (MG.)	PROTEOLYTIC ACTIVITY	
					RELATIVE ACTIVITY*	ACTIVITY PER GM.†
1	Control	3	76.3	101.7	1.01	2.05 ± 0.75‡
	Control hypo	4	48.7	62.1	0.59	1.97 ± 0.27
	Hypo + test	4	56.7	85.1	0.92	2.19 ± 0.26
	Hypo + thyroid	4	55.2	81.5	0.77	1.90 ± 0.27
	Hypo + test + thyroid	4	56.0	85.0	0.70	1.70 ± 0.17
2	Control	3	94.0	104.7	1.04	1.97 ± 0.19
	Control hypo	3	47.6	51.6	0.44	1.73 ± 0.27
	Hypo + test	3	70.6	94.5	1.06	2.18 ± 0.34
	Hypo + thyroid	3	58.3	74.5	0.74	1.98 ± 0.44
	Hypo + test + thyroid	3	57.6	60.0	0.56	1.93 ± 0.11
4	Control	3	128.0	145.8	1.93	2.68 ± 0.64
	Control hypo	2	71.5	81.2	0.50	1.25 ± 0.03
	Hypo + test	3	62.6	84.6	0.62	1.49 ± 0.07
	Hypo + thyroid	3	69.7	77.3	0.61	1.64 ± 0.25
	Hypo + test + thyroid	3	76.0	82.5	0.71	1.73 ± 0.54
8	Control	4	168.0	194.5	4.48	4.71 ± 0.87
	Hypo + test	4	79.0	75.1	0.66	1.81 ± 0.05
	Hypo + thyroid	4	72.7	73.0	0.78	2.29 ± 0.33
	Hypo + test + thyroid	4	74.7	90.0	1.08	2.65 ± 0.99

*Expressed as the ratio between the color produced by the total gland and the color of the tyrosine standard.

†Expressed as the ratio between the color produced per gram of gland tissue and the activity of the tyrosine standard.

‡Standard deviation.

glands, induced by the administration of desiccated thyroid, is accompanied by a corresponding increase in the proteolytic activity of these glands. Similarly, the suppression of these tubules by propylthiouracil results in a concomitant suppression of proteolytic activity. Although desiccated thyroid is capable of stimulating the salivary glands and producing an apparent premature maturation, the same substance is incapable of this activity in the absence of the pituitary gland. Furthermore, while it has been reported that thyroxine and testosterone administered simultaneously are capable of restoring the salivary glands to normal, the present studies indicate that these two compounds, administered alone or together, do not induce the degree of proteolytic enzyme activity or a histologic picture in hypophysectomized female animals comparable to that of control unoperated animals. Testosterone alone is completely ineffective although it does appear to potentiate the effects of thyroxine.

It appears that in the absence of the pituitary glands, some factor other than thyroid hormone or testosterone is necessary for the normal maturation of the rat submaxillary glands as judged by histologic and proteolytic studies under the conditions of this experiment. It is entirely possible that this may be some known or, as yet, unrecognized pituitary factor although it should be pointed out that a number of well-known pituitary hormones have not been excluded or examined for action.

SUMMARY

It has been shown that the proteolytic activity of the rat submaxillary gland is increased by the administration of desiccated thyroid and decreased by propylthiouracil. This alteration in activity occurs concomitantly with histologic changes in the gland.

Hypophysectomy prevents the morphologic development of this gland and in addition suppresses the proteolytic activity. This could not be prevented by the administration of either desiccated thyroid or testosterone alone or in combination.

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SURFACE CHANGES IN ENAMEL*

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THE GENERALLY accepted clinical observation that resistance to dental decay increases with tooth age has encouraged comparisons of some properties of enamel from old and young teeth. That no differences have been established between the inorganic content, microhardness, density, or x-ray density make it seem unlikely that significant changes occur throughout life in the major inorganic constituents of the enamel. However, indications that there may be age changes in the amount or nature of the organic components of enamel can be deduced from a few reports. For instance, Murray and Bowes[1] found more nitrogen in pyorrhetic (old) teeth than in young teeth and Chase[2] observed that more organic material remained after decalcification of older teeth as compared with younger ones. Beretta[3] noted that there is an increase in the thickness of the enamel cuticle with age. Whether changes occur in the nature as well as the quantity of organic material is not clear. Evidence suggesting this may be found in decalcified enamel sections in which the outer portions often show different structural and staining properties from the remainder of the organic structures (Manly,[4] Bibby,[5] Nishmura[6]). It has been suggested that such differences may be the result of the deposition of salivary proteins or other organic materials, such as bacterial products, in the enamel surface (Malleson,[7] Bibby,[8] Manly,[4] and others). That acid resistant material is in fact deposited in enamel is indicated by Beust's[9] and Enright and Friesell's[10] observations on areas of nonprogressive or arrested caries. Bibby[8] has suggested that the brown pigmentation occurring in the external layers of the enamel represents an acquisition of organic material which increases with the age of teeth.

The possibility that this is the case has never been adequately investigated. Therefore, as an introduction to a fuller consideration of the organic materials in the outer enamel, it seemed worth while to conduct a systematic survey of the differences in the extent of superficial enamel pigmentation in relation to tooth age.

MATERIAL AND METHODS

To facilitate comparison between teeth, only premolars with known history of previous oral environment of the teeth were used. These were obtained from the surgical department of the Eastman Dental Dispensary and

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from the offices of several dentists in Rochester, N. Y. As soon as possible after extraction, the teeth were numbered and scraped with dental scalers. Debris, calculus, and plaques were removed by brushing with an engine brush and pumice. After temporary storage in distilled water, the teeth were divided into 7 groups as follows: (a) no exposure, unerupted teeth, (b) 0-4 years, (c) 5-10 years, (d) 10-20 years, (e) 20-30 years, (f) 30-40 years, and (g) more than 40 years' exposure in the mouth.

Thereafter, a complete description of each tooth was recorded. This included the position in the mouth, its posteruptive age, surface changes (including intensity of brown pigmentations), and their position on the tooth surfaces. Using hydrocolloid impressions, white stone models were made of all teeth and surface changes recorded thereon with colored pencils. Prior to sectioning, the teeth were subjected to examination under a wide-field binocular microscope, and a further description of each changed or pathologic area noted on a specially prepared record card (Fig. 1).

Name	Case No.	Age
No. of Tooth	Early Caries (a) Location	
Chronological Age	(b) Size (c) Decalcification Area	
Surfaces with Brown Spots	Radiological Evidence	
Intensity of the Spots	Age of Caries	
Area Occupied	Caries of Adjoining Teeth	
Sound Surfaces	Hypoplasia	

Fig. 1.—History card.

Some teeth were placed in fixatives prior to sectioning, but since this was found to have no advantage most were stored in water.

Preparation of Ground Sections.—As soon as possible after the tooth record was completed buccolingual and mesiodistal ground sections were prepared from each tooth. The tooth was mounted in the desired orientation in the specimen holder of the cutting machine in dental plaster. Cutting was done under water on a 30-gauge silicon bonded carbide cutting wheel onto which the specimen was then gently raised by means of hand pressure. Using a rubber cork and hand pressure, the sections were ground under an adequate supply of water to desired thickness on graded abrasive wheels. Final polishing was done on both surfaces on the smooth Arkansas stone-wheel to give a section approximately 100 microns thickness. When the polishing was completed, the sections were washed with distilled water, dehydrated in alcohols, cleared in xylene and mounted in Bioloid.

Decalcification of Sections.—After study, some of the ground sections were subjected to carefully controlled decalcification under the microscope. The apparatus used is illustrated in Fig. 2. It consists of 2 capillary tubes which lead to the enamel surface of the tooth section fixed under a wax-sealed coverslip to a plastic slide with Plexi-glue. One of the tubes is connected by means

of polyethylene plastic tubing (100 gauge) through a two-way stopcock to reservoirs containing acid (1 per cent acetic buffered at pH 4.5), or alkali. The other tube provides an outlet for used fluids. By manipulating a screw clamp on the plastic tubing, the flow of acid was maintained at 30 drops a minute which causes visible decalcification in 40 to 60 hours in most sections. When desired, sections were dehydrated, cleared, and mounted in the usual way.

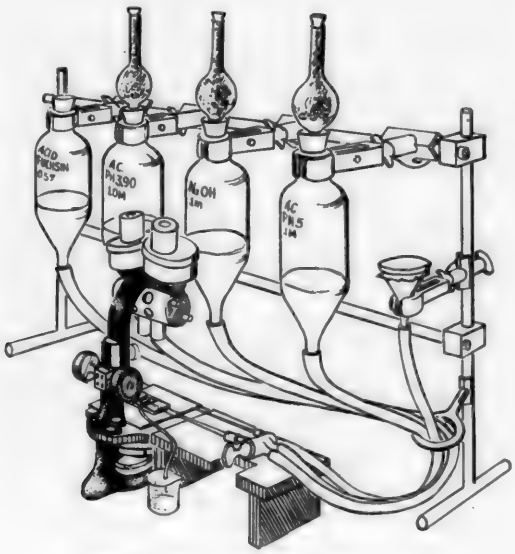

Fig. 2.—Decalcification apparatus.

Examination of Sections.—Because enamel surface pigmentation was being studied in relatively thick sections with beveled edges, a critical microscopic procedure was of utmost importance. This was achieved by careful attention to focusing on the enamel edge and by using a fine beam of light reducing diffraction and cross-illumination effect at the surface to a minimum. Low power (×100) was used for microscopic observation. To safeguard against biased interpretation, the sections were examined randomly without knowledge of the tooth under study.

In recording pigmentation in the enamel of the ground sections, clean nonpigmented enamel (Fig. 3) was used for reference. For each tooth, the gingival, middle, and occlusal thirds of the enamel were examined separately and for each the width of any pigmentation was recorded as a fraction of the enamel thickness. The degree of pigmentation was noted as heavy (+++), medium (++), or light (+) for each area. The observations were tabulated on prepared record forms.

In addition to the surface pigmentation, with which this study is concerned, varying amounts of other types of enamel pigmentation were also observed. These were islands of pigmentation in the body of the enamel and generalized pigmentation of the gingival area. Once these were recognized

they could be distinguished with certainty under the microscope. Color differences under fluorescent microscopy gave additional evidence of the differences between these and the surface type of pigmentation. Only the enamel surface pigmentation will be considered further in this report.

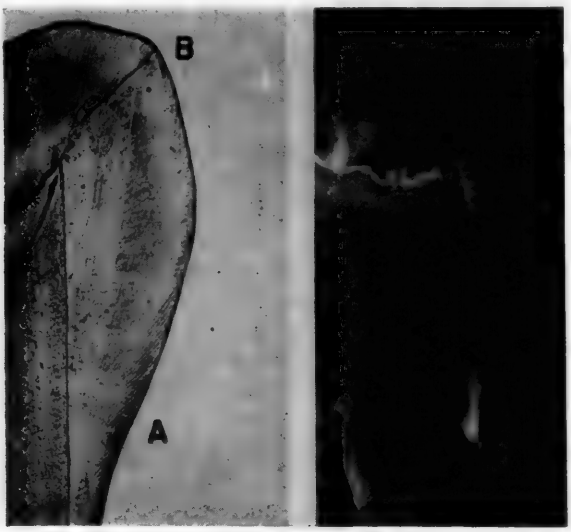

Fig. 3.—*Left,* Unstained ground section of enamel. Posteruptive age, 6 months. (Orig. mag. ×25.) *A-B,* Clean enamel surface in critical focus.
Right, Fluorophotomicrograph of a clean enamel surface. (Note the absence of fluorescence along the enamel surface.)

OBSERVATIONS

Typical surface pigmentation is shown in Figs. 4 and 5. It generally appears as a fine brown-pigmented layer in the surface enamel. Sometimes this shows as a structureless irregular layer, but at other times isolated groups of rods look as if they had been consolidated by the penetration of a brown-stained material while the adjacent rods appear normal. Under a strong ultraviolet light[11] the pigmented areas emit a bright bluish fluorescence (Fig. 6).

Table I summarizes the findings on pigmentation of the enamel surface at various ages. There is an absence of pigmentation on the occlusal third of all surfaces in the unerupted teeth and in those teeth with 4 and 10 years' postoperative age. However, with increasing age, greater amounts of pigmentation are noted. In the buccal surface, for instance, the pigmentation represents on the average 0.07 of the enamel thickness in the 10- to 20-year group; 0.1 in the 20- to 30-year group; 0.19 in the 30- to 40-year group; and in the over 40-year group 0.22 of the enamel.

In the middle third of the enamel there is also a tendency toward an increase in the extent and intensity of surface pigmentation with age (Table I).

The pigmentation of the gingival third of the enamel (Table I) also shows an increase in the amount and intensity of pigmentation with increasing age, going from yellowish brown in the younger teeth to brownish black

Fig. 4.

Fig. 5.

Fig. 4.—Unstained ground section of enamel, posteruptive age, 31 years. (Orig. mag. ×25.) *A-B*, Surface pigmentation of the enamel.

Fig. 5.—Ground section of enamel, posteruptive age 48 years. (Orig. mag. ×25.) *A-B*, Surface pigmentation of enamel. *C-D*, Optical artifact.

Fig. 6.—Shows the fluorescence of surface pigmentation along the enamel surface.

TABLE I
QUANTITATIVE ANALYSIS OF SURFACE PIGMENTATION AT VARIOUS AGES

ERUPTION AGE IN YEARS	NUMBER OF TEETH		BUCCAL	LINGUAL	MESIAL	DISTAL
Unerupted	11	*occ.	0	0	0	0
		mid.	0.09 (0 . . . +)	0.10 (0 . . . +)	0	0.10 (0 . . . +)
		ging.	0.75 (0 . . . +++)	Complete pigment	0.50 (0 . . . +)	Complete pigment
0- 4	14	occ.	0	0	0	0
		mod.	0.05 (0 . . . +)	0.07 (0 . . . +)	0.05 (0)	0.05 (0)
		ging.	0.50 (0 . . . ++)	0.50 (0 . . . ++)	0.50 (0 . . . ++)	0.50 (0 . . . ++)
5-10	14	occ.	0	0	0	0
		mid.	0.08 (0 . . . +)	0.05 (0 . . . +)	0.05 (0 . . . +)	0.07 (0 . . . +)
		ging.	0.70 (0 . . . +++)	0.50 (0 . . . ++)	0.50 (0 . . . ++)	Complete pigment
10-20	11	occ.	0.07 (0 . . . +)	0.08 (0 . . . +)	0.07 (0 . . . +)	0.08 (0 . . . 4)
		mid.	0.10 (0 . . . ++)	0.10 (0 . . . ++)	0.10 (0 . . . +)	0.15 (0 . . . +)
		ging.	Complete pigment	0.70 (0 . . . ++)	0.70 (0 . . . ++)	Complete pigment
20-30	10	occ.	0.10 (0 . . . ++)	0.07 (0 . . . ++)	0.10 (0 . . . ++)	0.11 (0 . . . ++)
		mid.	0.12 (0 . . . ++)	0.15 (0 . . . ++)	0.16 (0 . . . ++)	0.15 (0 . . . ++)
		ging.	0.60 (0 . . . ++)	0.77 (0 . . . +++)	0.70 (0 . . . ++)	0.70 (0 . . . +++)
30-40	10	occ.	0.19 (0 . . . ++)	0.17 (0 . . . ++)	0.12 (0 . . . ++)	0.12 (0 . . . ++)
		mid.	0.25 (0 . . . ++)	0.20 (0 . . . ++)	0.20 (0 . . . ++)	0.16 (0 . . . ++)
		ging.	0.75 (0 . . . +++)	Complete pigment	Complete pigment	Complete pigment
40-over	12	occ.	0.22 (0 . . . ++)	0.22 (0 . . . ++)	0.20 (0 . . . ++)	0.15 (0 . . . ++)
		mid.	0.25 (0 . . . ++)	0.20 (0 . . . ++)	0.18 (0 . . . +++)	0.16 (0 . . . ++)
		ging.	Complete pigment	Complete pigment	Complete pigment	Complete pigment

Key: 0.07 or 0.10 = extent of surface pigment penetration.
(0 . . . +) or (0 . . . ++) = range of color intensity of pigmentation.

*Occlusal third (occ.) middle third (mid.) and gingival third (ging.) is given for each age group and surface.

in the older teeth. It is interesting to note that the pigmentation in the gingival region was present in all the teeth examined and that it gave a different type of fluorescence from that given by the pigmentation elsewhere on the surface (Fig. 7).

The decalcified sections show that the surface pigmentation is highly resistant to the action of various acids. Moreover, a larger residue is left after decalcification of sections showing surface pigmentation than the ones without pigmentation.

DISCUSSION

This study revealed a rather definite increase in surface pigmentation with age. This finding of itself provides a satisfactory explanation for the clinical observation that browning of teeth occurs with age. The demonstration (Fig. 7) that the pigmented material is acid resistant may account for the higher resistance of old teeth to dental decay.

Although this study was not directed toward determining the nature of the pigmentation observed in the surface enamel, some consideration should be given as to what type of change the increased pigmentation represents. Under the microscope it looks as if impregnation of the enamel rod sheaths or

inter-rod material had been increased by addition of the pigmented material. This sequence of change would be in keeping with what could be expected under the influence of decalcification or caries, and suggests that the process of decalcification which might have led to caries has in some way become deviated and that, instead of continuous loss of inorganic material, there has been a replacement of the lost material by this pigmented substance.

Fig. 7.—Unstained decalcified enamel section, posteruptive age, 40 years. (Orig. mag. ×25.) *A-B*, Pigmented areas on the enamel surface which resist the action of acids. *C*, Partially decalcified areas. *D*, Completely decalcified area. (Note the surface outline of enamel.)

Little can be offered about the nature of the pigmentation from our study. The fact that the pigmented areas leave a greater amount of residue on acid decalcification suggests that they have an increased organic content or may be composed largely of organic material.* Further reasons for believing that we are dealing with something in the nature of an increase or additional deposition of organic material may be implied from the assumption that this increased pigmentation which we have seen in old teeth will account for the increased recovery of surface-film material found by Chase.[2] This conclusion also fits in with the finding of Murray and Bowes[1] who demonstrated increased organic material in older teeth. Actually, such an increase of surface organic material would account for many of the earlier observations in the dental literature, such as the thickening of the cuticle seen by Leber and Rottenstein,[12] the accretions observed by Miller,[13] Berreta's thickening of the

*Subsequent work by the senior author has demonstrated that an increase in the nitrogen content of enamel, both on weight and volume basis, parallels the age increase in pigmentation.

cuticle, Hopewell-Smith's[14] pigmentation of the surface membrane and Malleson's[7] observation of increased surface pigmentation and his suggestion that this could be accounted for by protein material entering the enamel from saliva. In short, there are many suggestions in the dental literature which support the possibility that with increasing age there is an acquisition of organic material by the enamel surface.

Because of the low or negligible metabolism of the enamel, it is hard to conceive of acquisition of organic material by enamel except from the outside of the tooth. There are 2 possible external sources of organic material: (1) Bacteria or debris lodging in the enamel defects and undergoing a consolidation of the sort suggested by Bibby.[8] This possibility receives some support from Chase's[15] demonstration that surface pellicles are formed on extracted teeth placed in saliva or bacteria containing liquid, and (2) the suggestion (by Malleson[7]) that the constituents of saliva enter the enamel and are deposited there. Of course, both processes may take place and contribute to varying extents to the formation of organic deposits in different teeth or even different portions of the same tooth.

SUMMARY

1. A histologic study of brown pigmentation in enamel surfaces in relation to tooth age has been made on ground sections from 82 teeth.

2. Microscopic study of predetermined areas of gingival, middle, and occlusal thirds of enamel surfaces showed a consistent increase in the amount and intensity of surface pigmentation with increasing age.

3. Decalcification under the microscope of some sections demonstrated that the pigmented areas are highly resistant to the action of acids.

4. It is suggested that the material making up the pigmented areas is basically organic in nature having its origin in the saliva.

The authors wish to acknowledge the assistance and cooperation of Mr. George Dickson, Dr. A. F. Forziati, and Mr. M. E. Lawson in the work dealing with the fluorescent properties of enamel.

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HISTOLOGIC CHANGES IN THE INCISOR TEETH OF RATS SERIALLY SACRIFICED AFTER RECEIVING 1,500 R OF 200 KV. X-RAY IRRADIATION

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EXPERIMENTS by English, Schlack, and Ellinger[1] on the effects of a single dose of radiation on growing incisor teeth of white rats examined a relatively long time following exposure (100 days) indicated striking changes in the developing incisor teeth. The teeth usually showed a definite break in continuity, such that 2 segments were formed: one segment representing the portion of the tooth which was present at the time the animal was irradiated, the other being the portion of tooth formed following exposure. In the maxilla, the first segment frequently was lost at 100 days leaving a stumplike tooth; in the mandible, the second-formed segment frequently grew laterally to the first, producing in effect an "extra" incisor tooth. As a result, many rats appeared to have 4 lower incisor teeth, whereas upper incisor teeth were short or "missing."

The purpose of this study was to observe histologic changes in the incisor teeth of rats exposed to the same dose of radiation but sacrificed at various intervals beginning soon after exposure (16 hours) and continuing as long as animals survived. It was planned to observe the locus of damage and attempt to determine the point from which the "second" tooth originated.

MATERIALS AND METHODS

One hundred weanling white rats of a strain developed at the Naval Medical Research Institute (originally Osborne-Mendel) were used in this experiment. The age range of the animals was from 21 to 23 days at the time of exposure. Fifty animals were given head radiation; 50 littermates, paired by sex and weight, were retained as controls (Table I). All animals were fed Rockland rat diet, pellet form, ad libitum.

The equipment used for irradiating the rats was a standard Picker commercial x-ray machine. The physical factors used were 200 KVP, 20 ma., filters ¼ mm. copper and 1 mm. aluminum, half-value layer 0.82 mm. copper,

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The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or as necessarily reflecting the views of the Navy Department or the naval service at large.

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TABLE I

DATA CHART FOR ONE HUNDRED IRRADIATED AND CONTROL WHITE RATS

LITTER NUMBER	EXPERIMENTAL RAT NUMBER	SEX	SACRIFICED OR FOUND DEAD	CONTROL RAT NUMBER	SEX	SURVIVAL TIME IN DAYS
558	1929	M	F D	1933	M	8
558	1930	F	F D	1934	F	8
558	1931	M	SAC	1935	M	7
558	1932	F	SAC	1936	F	7
560	1937	M	SAC	1939	M	24 Hours
560	1938	F	SAC	1940	F	24 Hours
564	1941	M	SAC	1943	M	24 Hours
564	1942	F	SAC	1944	F	24 Hours
566	1945	M	SAC	1947	M	8
566	1946	F	F D	1948	F	8
568	1949	M	SAC	1951	M	48 Hours
568	1950	F	SAC	1952	F	48 Hours
570	1953	M	F D	1955	M	8
570	1954	F	F D	1956	F	9
572	1957	M	SAC	1959	M	7
572	1958	F	SAC	1960	F	7
573	1961	M	F D	1963	M	8
573	1962	F	SAC	1964	F	11
574	1965	M	SAC	1967	M	48 Hours
574	1966	F	SAC	1968	F	48 Hours
599	1969	M	SAC	1971	M	3
599	1970	F	SAC	1972	F	3
577	1973	M	SAC	1975	M	16
577	1974	F	F D	1976	F	8
580	1977	M	F D	1979	M	8
580	1978	F	F D	1980	F	8
584	1981	M	F D	1983	M	8
584	1982	F	F D	1984	F	8
589	1985	M	F D	1987	M	9
589	1986	F	F D	1988	F	8
560	1989	M	F D	1993	M	8
560	1990	F	SAC	1994	F	8
560	1991	M	SAC	1995	M	7
560	1992	F	SAC	1996	F	7
593	1997	M	SAC	2001	M	5
593	1998	F	SAC	2002	F	5
593	1999	M	SAC	2003	M	5
593	2000	F	SAC	2004	F	5
597	2005	M	SAC	2087	M	21
597	2006	F	SAC	2088	F	21
594	2007	M	F D	2013	M	8
594	2008	F	F D	2014	F	8
594	2009	M	F D	2015	M	8
594	2010	F	F D	2016	F	8
594	2011	M	F D	2017	M	8
594	2012	F	F D	2018	F	8
603	2019	M	SAC	2023	M	16 Hours
603	2020	F	SAC	2024	F	16 Hours
603	2021	M	SAC	2025	M	39 Hours
603	2022	F	SAC	2026	F	39 Hours

target-skin distance 50 cm. The exposure time required for a dose of 1,500 r under the physical conditions used was 34 minutes, 5 seconds (44 r per minute). Animals were exposed in groups of 10, using a 20 by 20 cm. cone.

In order to restrain the movement of rats during radiation and to be sure that only their heads would be exposed, they were placed in plastic boxes that had separate compartments for each animal. The heads of the animals were held in a fixed position by a tight-fitting, built-in neck stock, so that it was impossible for the head to be withdrawn from the field of exposure or for the body to be thrust from under the protection of the lead shield.

After radiation the animals were maintained under constant conditions of temperature and humidity. Beginning at 16 hours following radiation and continuing at 24, 39, and 48 hours and 3, 5, 7, 8, 9, 11, 16, and 21 days, the animals were sacrificed and the parts to be studied were placed immediately in 10 per cent neutral formalin.

The heads were severed by a midline incision and a radiograph was made of each side. The hard tissues were decalcified in formic acid and sodium citrate, embedded in paraffin or celloidin, and serial sections were made of the tissues. In each rat, sagittal sections were made of 1 maxilla and 1 mandible, and the other mandible was cut in cross section. All of the sections were stained with hematoxylin and eosin.

RESULTS

Twenty of the 50 experimental animals did not survive the experiment and died apparently as a result of the radiation procedures. Eighteen of the 20 animals died on the eighth postradiation day; the remaining 2 were found dead on the ninth day (see Table I). This 40 per cent mortality rate should not be interpreted as a true figure for the group because several of the sacrificed animals might have died from the results of the radiation if they had not been sacrificed. This was particularly true in some cases in which an animal obviously was moribund and was sacrificed immediately.

Each of the control animals survived the experiment until it was sacrificed to coincide with the sacrifice (or radiation death) of its corresponding experimental animal. Ten of the experimental animals that were found dead were females, and 10 were males.

The experimental and control animals selected for histologic study lived 16 and 24 hours and 5, 7, 8, 9, 16, and 21 days after exposure. All of these selected animals were sacrificed with the exception of the 9-day experimental animals which had been found dead. Consideration now will be given the sections examined according to the postradiation time.

Sixteen Hours and Twenty-Four Hours.—Histologic examination of sagittal sections of the right mandibular and maxillary incisors of animals sacrificed 16 and 24 hours following exposure revealed no significant deviations from the normal (Figs. 1 and 4 show normal). The cross sections of the left mandible also revealed what was considered to be within normal limits.

Fig. 1.—Sagittal section of mandibular incisor tooth of nonradiated rat (Orig. Mag. ×50), stained with hematoxylin and eosin. The apical end of the developing tooth is shown, the apex being to the left. Letters mark the following structures: *E* = enamel, *D* = dentin, *A* = ameloblasts, *O* = odontoblasts, *P* = pulp, *B* = bone.

Fig. 2.—Sagittal section of mandibular incisor tooth taken 5 days following radiation. Location, staining, and lettering are as in Fig. 1, with additional markings: *X* = reduction of labial odontogenic epithelium into a flattened strand, *C* = cystic spaces and edema in apical area, *Z* = osteodentin formation at apical end of odontoblastic layer.

Five Days.—Histologic examination of teeth in the animals sacrificed 5 days following exposure revealed numerous alterations. The changes were characterized principally by a marked disturbance in amelogenesis and dentinogenesis. It was noted that only the areas undergoing development at the time of exposure appeared to be affected. Sagittal sections of the right mandibular incisor (Fig. 2) revealed no significant alterations in the ameloblastic layer of those mature, pre-exposure portions of the tooth where enamel already had been formed. There was a marked disturbance in the ameloblastic epithelium toward the formative end of the tooth. Here the ameloblastic layer lost its tall, columnar appearance and became low, cuboidal to flattened, fading into the connective tissue stroma so that it could no longer be recognized.

The disturbance in dentinogenesis was also severe, as dentin formation appeared to cease. The odontoblastic layer appeared undisturbed in the incisal pre-exposure segment of the tooth, but as the odontoblasts approached the formative end of the tooth, they lost their tall, columnar appearance and became smaller and darker with deep-staining nuclei. It was at this point near the area of active tooth formation that these cells appeared to have become enmeshed in a pale-staining, eosinophilic, amorphous material. This material somewhat resembled bone and is, hereafter, referred to as "osteodentin" (Fig. 3). No changes other than the formation of osteodentin were observed in the dental pulp, and that portion of the pulp lying in the pre-exposure segment was completely normal.

The connective tissue between the odontogenic area and the bottom of the alveolus revealed a slightly edematous appearance and contained moderately large, thin-walled vessels engorged with red blood cells. These changes are the vascular changes of the inflammatory reaction, but no inflammatory cells, such as neutrophils, lymphocytes, and plasma cells, were noted.

Serial cross sections made of the left mandibular incisor revealed a loose connective tissue stroma containing large vacuolated cystlike spaces in the area normally occupied by the developing incisor. Centrally, there was one strand of odontogenic epithelium composed of low cuboidal, densely staining cells with a large nuclear-cytoplasmic ratio. Sections taken farther incisally (Fig. 5) revealed the beginning of osteodentin formation adjacent to flattened odontoblastic cells. At this point, the ameloblastic layer remained flattened. The characteristic, inverted U-shape of the developing incisor was apparent here. Sections taken still farther incisally revealed that the ameloblastic layer assumed its normal, tall, columnar appearance with enamel formation (Fig. 6). In place of a normal odontoblastic layer, osteodentin was present which extended well within the pulp chamber. There was a thin layer of dentin present in association with the enamel.

As sections approached the pre-exposure incisal end of the tooth, they did not differ from normal controls (Fig. 4). Sagittal sections of the maxillary incisors revealed the same kind of changes found in the mandibular incisor.

Seven Days.—All of the changes that were noted in the 5-day group also were present in the 7-day group, however, there was one important difference

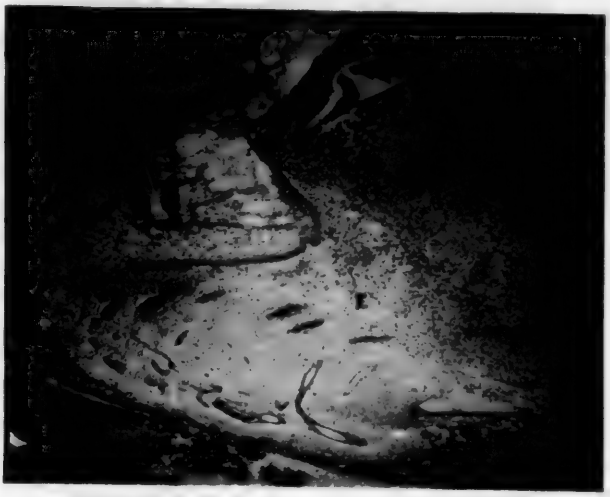

Fig. 3.—Sagittal section of maxillary incisor taken 5 days after radiation (Orig. Mag. ×50), hematoxylin and eosin stain. Note particularly large mass of osteodentin (*O*), atrophic odontogenic epithelium (*X*), edema (*E*), and large vascular spaces (*V*).

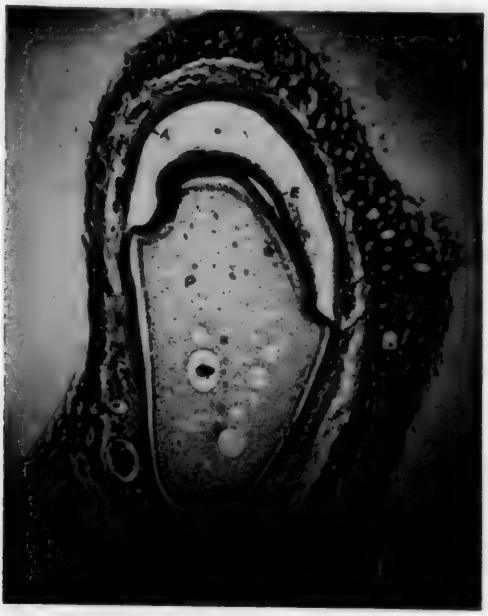

Fig. 4.—Cross section of mandibular incisor tooth of nonradiated rat (Orig. Mag. ×50), hematoxylin and eosin stain. Letters indicate following: *A* = ameloblastic layer, *E* = enamel, *D* = dentin, *O* = odontoblastic layer, *P* = pulp, *B* = bone.

Fig. 5.—Cross section of mandibular incisor tooth taken 5 days following radiation (Orig. Mag. ×50), hematoxylin and eosin stain. Note edema (*E*), osteodentin (*Z*), and atrophic odontogenic epithelium (*X*).

Fig. 6.—Same as Fig. 5, section taken further incisally. Note tall columnar ameloblasts. (*A*), enamel matrix (*E*) and osteodentin (*Z*).

Fig. 7.—Sagittal section of mandibular incisor tooth taken 7 days following radiation (Orig. Mag. ×50), hematoxylin and eosin stain. Although there is considerable alteration, similar to that observed in the 5-day rat, there is now seen a new area of proliferation of odontogenic epithelium (*P*). Note the connecting band of atrophic epithelium (*X*). Compare distance from (*O*) to (*P*) with same distance in Fig. 8.

Fig. 8.—Sagittal section of mandibular incisor tooth taken 9 days following radiation (Orig. Mag. ×50), hematoxylin and eosin stain. The tooth is divided distinctly into a pre-exposure (*O-A*) and postexposure (*P-B*) segment. The postexposure segment shows predentin formation (*D*) and epithelial proliferation (*P*). Hemorrhage (*H*) is present.

noted at the most apical portion of the odontogenic epithelium. Here, a bulbous process of epithelium had formed and was connected to the atrophic epithelium noted in the 5-day group (Fig. 7). The cells of this newly proliferating epithelium were similar to those seen at the most apical portion of normally developing incisors, but they had not yet begun to differentiate into cells that resembled ameloblasts. The proliferating epithelium was connected to the pre-exposure segment by a thin, flattened band of tissue. Around this mass of odontogenic epithelium there was a condensation of mesenchymal cells which seemed to be differentiating into odontoblasts. A small amount of eosinophilic material was present in association with these odontoblasts.

Eight Days.—By the eighth day one could observe a further proliferation and differentiation of the epithelial cells into ameloblasts at the basal end of the newly formed mass of cells.

Nine Days.—Sections of the 9-day group revealed even further development (Fig. 8) of what are now obviously regenerating tooth-forming tissues. The degenerative processes noted in the previous groups (except 16 and 24 hours) again were present, apparently having taken place in areas undergoing active development at the time of exposure. The incisor tooth structure now may be divided distinctly into 2 segments; (1) the incisal pre-exposure section, and (2) the newly formed section at the apical end of the tooth socket. The newly formed portion had developed enough that predentin could be seen. No evidence of enamel matrix formation was noted at this stage. Serial cross sections at this location revealed a connecting band of atrophic epithelium between the pre-exposure and postexposure segments. It consisted of 2 rows of thin, flattened cells.

Sixteen Days.—Tooth formation in 2 segments again was evident at 16 days. In sections examined, a connection between the 2 was apparent, consisting of a bridge of irregular dentin (Fig. 9). The second-formed segment was composed of structures that fairly well resembled normal tooth development. The surrounding periodontal membrane contained numerous, large, hemorrhagic areas.

Twenty-One Days.—At 21 days the second-formed segment was well established and did not differ greatly from earlier sections, except the pre-exposure segment had erupted further incisally making room for the developing tooth. It was noted that the damage to the areas under development at the time of exposure was still evident at 21 days. It also was noted that the second-formed segment presented some irregularities in its form, although, in general, it fairly well resembled a normal developing tooth.

DISCUSSION AND CONCLUSIONS

Cause of Gross and Microscopic Changes.—Since none of the control rats manifested any significant gross or histologic deviations from the normal and since all of the rats that showed pathologic alterations had been subjected to

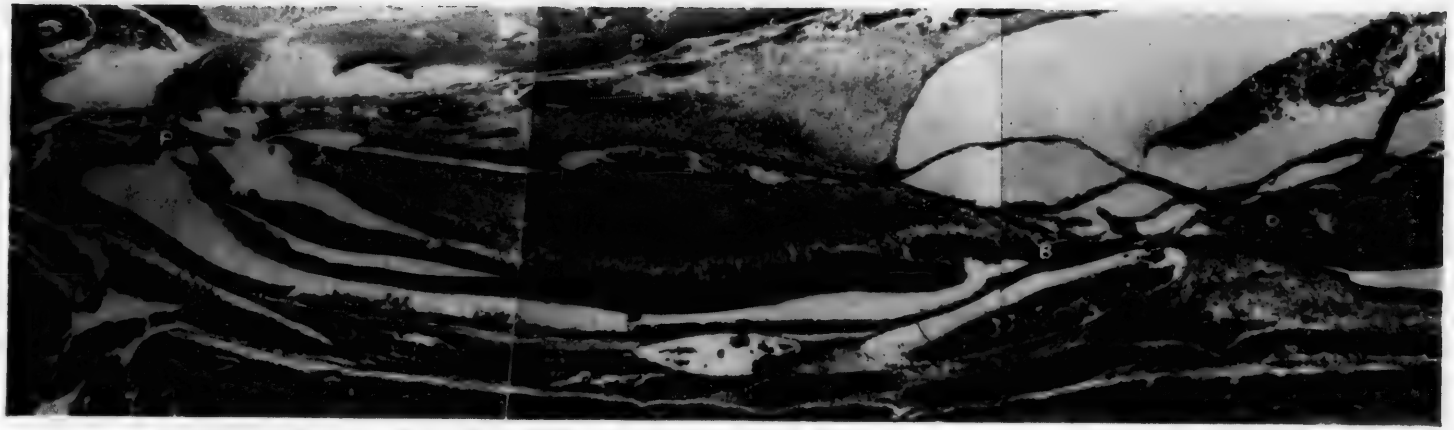

Fig. 9.—Sagittal section of mandibular incisor tooth taken 16 days following radiation (Orig. Mag. ×50). hematoxylin and eosin stain. The postexposure segment is now quite long (*P-B*), thereby increasing the distance from the proliferative area (*P*) and the pre-exposure tooth (*O*). In another section of this same tooth a band of osteodentin (*O*) connected the two segments.

x-ray irradiation, it was presumed that the changes seen were a result of the radiation procedures. This assumption is also substantiated by previously reported work in which rats were subjected to identical procedures.[1]

Earliest Evidence of Radiation Injury.—Alterations from the normal were not observed in microscopic examinations of those animals that were sacrificed 24 hours or less following exposure. It is possible that if larger numbers of animals had been used, some might have revealed microscopic evidence of damage about the incisor teeth. A lack of visible alterations does not mean, however, that damage did not occur. To the contrary, severe damage did take place as evidenced by the appearance of teeth examined a few days later. This lack of early visible, pathologic alterations is not inconsistent with the findings of others in the field of x-ray irradiation. In some instances[2-5] such a delay is called a "latent effect." In the case of developing teeth, one can consider the structural detail of the tooth which is being formed as a permanent record of the functional capacity of the odontogenic cells.

The first evidence of injury appeared 5 days following exposure to the x-rays. The changes observed were severe, which would indicate there might have been alterations visible had specimens been examined between the 24-hour and 5-day period. Until further studies are done, we can say only that the first visible damage occurs between the first and fifth days.

It should be remembered that different strains of animals may show substantial differences in susceptibility to radiation, and that a change in the conditions of radiation will result in an unpredictable change in the biologic response.

Relative Radiosensitvity of Dental Structures.—The first histologic section that demonstrated visible microscopic changes revealed alterations in both ameloblasts and odontoblasts. Therefore, from this study it is impossible to state which structure was first to show tissue damage. Even if this were determined, it would not necessarily follow that the earlier was more sensitive to x-radiation because the "latent period" may be different for various tissues. This, perhaps, explains why various authors disagree as to which are the more radiosensitive cells; ameloblasts or odontoblasts.

Locus of Damage.—Although it is impossible to state whether the ameloblasts or the odontoblasts were the more sensitive, one can state that retrogressive changes took place in each and that the locus of damage was at the most apical end of the developing tooth, where the cells were less differentiated and more metabolically active. It was apparent throughout all the sections of the radiated animals that the more mature, older, differentiated ameloblasts and odontoblasts lacked the histologic alterations seen in the less differentiated, younger, and more physiologically active cells located near and at the formative end of the tooth.

The ameloblastic layer appeared thinner and more atrophic as the formative end of the tooth was reached until a thin, flattened band of epithelium was all that remained. The odontoblastic layer also was altered (osteodentin

formation) as the formative end was reached, eventually disappearing completely. The structures of pre-exposure portions of the teeth, which were already formed, did not reveal any significant alterations.

Actually this is what one would expect if the law of Bergonie and Tribondeau is true; that is, the radiosensitivity of a tissue is proportionate to its reproductive capacity and inversely proportionate to its degree of differentiation.[6] The observation that odontoblastic activity ceases altogether may be attributed to damage to the odontogenic epithelium; that is, the ameloblasts were injured sufficiently so that they no longer had an organizing effect upon adjacent mesenchymal cells. Therefore, even if the odontoblasts were not affected directly, they may be altered indirectly since their development depends upon the organizing influence of the ameloblasts.[7]

Another factor in establishing the locus of damage is that severe alterations were seen at a distance from the bottom of the alveolus in direct proportion to the time following exposure to x-ray. In other words, the longer the time following exposure, the farther the damaged area of the tooth had erupted. Rough measurements by an ocular micrometer indicated an eruption rate similar to the findings of others.[7]

Re-establishment of Tooth Formation.—Our results clearly indicate that 6 days after radiation new odontogenic epithelium was formed and subsequently produced tooth structure. Since either these cells or their predecessors were in the area of irradiation, 1 or 2 possible events took place:

1. The undifferentiated predecessors received radiation and were damaged to some extent but recovered. It is possible that some cells were killed outright and others were damaged in moderate to severe degrees.
2. The undifferentiated cells were so resistant to radiation that they escaped damage.

The odontogenic epithelium, which appeared atrophic, probably was present at the time of irradiation. Then, following radiation, new odontogenic epithelium differentiated and revealed a more normal morphologic appearance and staining reaction. This area of newly developing tooth structure occurred at the apical area of the tooth and proliferated outwardly much in the same manner as normal epithelium. There are gradations in function of the odontogenic cells between those which are nonfunctioning and those which function normally. At one stage we have shown that an "osteodentin" material is formed, rather than normal tooth structure, and perhaps the odontoblasts which formed this material were just beginning to differentiate at the time of radiation.

New development in the apical area was noted first in the odontogenic epithelium. As the ameloblastic layer developed, differentiation of adjacent mesenchymal cells into odontoblasts soon followed. As the recovery of these tissues continued, it soon was apparent that a finely formed tooth structure was developing directly apical to the pre-exposure portion of the tooth.

X-ray irradiation, under the conditions used, caused severe degenerative changes in the odontogenic tissues. It would be interesting to subject animals to higher doses of radiation under the same conditions to determine what dose, if any, could cause irreversible damage to the anlage without causing death of the animal.

SUMMARY

An experiment was undertaken to observe changes in the incisor teeth of rats when exposed to a single dose of x-ray irradiation. It was planned to observe the locus of damage and to determine the point from which the "second tooth"[1] had originated.

Fifty white rats were irradiated locally to the head with 1,500 r of 250 kv. x-ray. These rats were serially sacrificed simultaneously with 50 littermate control rats at various intervals beginning at 16 hours following exposure.

Estimation of the histologic sections revealed that the locus of radiation injury was principally within the young, metabolically active, functioning odontogenic layers of cells. It appeared that, even though this damaged tissue underwent severe retrogressive changes, the irradiation was not sufficient to destroy either the undifferentiated epithelial and mesenchymal cells, or those odontogenic cells that had completed their physiologic function.

The "second tooth" was observed to arise from these surviving undifferentiated cells of the tooth germ. The reason there were 2 separate segments of tooth arising from a single tooth germ was because of a severe interruption in tooth formation following radiation, and during the early recovery period abnormal tooth substance was formed. Finally, relatively normal tooth structure was again formed by the odontogenic cells.

The authors gratefully acknowledge the valuable technical assistance of the following: Clarence Miller of the Naval Medical Research Institute for histopathologic and photographic work, Thomas R. Smith and Virginia L. Lux of the Naval Dental School for histologic technical work, Adelaide R. Horton of the Naval Dental School for preparation of the data chart; and to other members of the Dental Department, Naval Medical Research Institute for care of animals and general assistance.

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STUDIES ON THE DENTITION OF THE CYNOMOLGUS MONKEY

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THE purpose of this presentation is to describe the dentition of an Old World monkey, *Macaca cynomolgus,* and to comment upon the incidence of dental abnormalities. Thirteen skulls of this species were obtained at the end of poliomyelitis experiments through the courtesy of Dr. Gertrude van Wagenen, Department of Obstetrics, Yale University School of Medicine. An additional 4 skulls were available at the termination of experiments in our laboratories where the specimens had been maintained on purified diets[1] supplemented adequately with ascorbic acid.

EXPERIMENTAL

Upon termination of the experiments, the monkeys were sacrificed and their heads thoroughly fixed in a 10 per cent neutral formalin solution. Before examination, the skin and musculature were removed from the skulls.

First the occlusal patterns and orthodontic discrepancies of the dentitions were studied grossly. Then the jaws were examined under a binocular dissecting microscope for carious lesions and any superficially detectable morphologic abnormalities. Typical examples of monkeys of various age groups were photographed. Radiographs were taken of representative jaws.

Some specimens were prepared for histologic sectioning by dehydration in graded alcohols and passage into xylol. Each specimen was then placed in a suitable glass vial or jar and embedded in polymerized methyl methacrylate by the procedure described by Sognnaes, Shaw, Solomon, and Harvold.[2] When the polymerization was complete after about 10 days, the glass containers were broken. The polymer block, containing the specimen was mounted in plaster and cut into 1 to 2 mm. slices which later were reduced in thickness by grinding to the desired thickness on flat water-cooled, rotating, grinding stones. Sections were polished and then placed in methyl methacrylate monomer to dissolve the embedding medium. After clearing in xylol, the sections were mounted on glass slides and coverslipped.

The species, *Macaca cynomolgus,* is an Old World monkey of the division Catarrhini and the Cercopithecidae family. Seventeen skulls of this species were examined, of which 3 were adults with a full complement of permanent teeth. Three were young with fully erupted deciduous dentitions and 11 were in the mixed dentition stage with both deciduous and permanent teeth present. A total of 427 teeth were examined of which 253 were deciduous and 174 were permanent.

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The formula of the deciduous dentition was:

$$\text{I } \frac{2}{2} \text{ C } \text{ M } \frac{1}{1} \frac{2}{2} .$$

The formula for the permanent dentition was:

$$\text{I } \frac{2}{2} \text{ C } \frac{1}{1} \text{ Pm } \frac{2}{2} \text{ M } \frac{3}{3} .$$

Typical examples of the tooth and jaw forms of young monkeys are shown in Figs. 1 and 2 and of adult male and female monkeys in Figs. 3 and 4.

Bone structure seemed good in all 17 specimens with no evidence of osteoporosis or periodontal disease. No mobility of teeth was detected.

The usual occlusion in the specimens examined was the Angle Class I molar relationship, anterior edge-to-edge bite; all teeth were in proximal contact except canines with their second incisors, between which there was a space of several millimeters, with the maxillary space the larger of the two.

The form of the maxillary and mandibular dental arches was fairly similar; both have an anterior curvature from canine to canine with the posterior teeth in almost straight, approximately parallel, lines.

No crowding of the teeth was seen in the specimens examined. Only 2 of the monkeys had occlusal abnormalities; both monkeys were in the mixed dentition stage. One had a unilateral posterior crossbite. The other one which had been born and raised in our laboratory of parents maintained on a purified ration had a more severe orthodontic problem. Its molars were in Class I relation, but there was an anterior open bite, with a 3 mm. shift of the midline to the right. The lower right second premolar was in a crossbite relationship with its maxillary antagonists—right maxillary first and second premolars. The radiographs of this animal's upper and lower jaws are shown in Figs. 5 and 6.

Tooth Form.—The posterior teeth have steep, pointed cusps, the inclined planes joining at about right angles. The canines in the deciduous dentition were relatively small; only the maxillary deciduous canines extended slightly above the occlusal plane, with no conspicuous difference in size and shape between the sexes. However, the permanent canines were extremely well developed, especially in the male, where this tooth has a tusklike form.

Attrition.—Wear patterns were frequently seen in the specimens examined. The teeth of all monkeys in the mixed or adult dentition stage had some degree of attrition. Ten of the specimens had definitely pronounced facets and attrition with secondary dentin formation. Canines were the most common teeth involved, with the incisors next in frequency. Four monkeys in the mixed dentition stage had such severe attrition of the deciduous molars that all enamel was worn off the occlusal surface and brownish concavities in the dentin had been produced. Attrition in the mandibular incisors had led to pulp exposure and devitalization of the dental pulps in 2 animals.

Calculus.—The oral cavity was relatively clean in 16 of the 17 specimens examined. Only 4 of those showed slight accumulation of materia alba on the gingival third of the posterior teeth. However, one of the adult monkeys had a relatively large amount of calculus on the labial and buccal surfaces of all teeth. The gingiva looked somewhat swollen and there was loss of alveolar bone with exposed root bifurcations on the posterior teeth. In the remaining 16 specimens, the gingiva seemed firm, knife-edged, and well attached with no evidence of inflammatory processes.

Caries.—Upon examination of the intact teeth under the microscope, many of them revealed dark discolored areas in the pits and fissures of the posterior teeth. When a small sharp explorer was used to probe these areas, it was caught in the lesions and could be sunk slightly into them. The lesions were sufficiently small that they could not be easily detected during examination of the living animal. Radiographs did not reveal these lesions since none were so extensive as to have destroyed any appreciable amount of the tooth structure. Thus there was no duplication in these animals of the rampant caries of human subjects.

Upon examination of the plastic-embedded ground sections under the microscope, either no enamel was observable at the fusion points of developmental lobes or the thin enamel bridge, or, if present, was not normal in structure. In most cases, a dentin reaction was seen under the lesions that manifested itself as dead tracts.

Of the 17 specimens examined, only 5 were caries-free—all 3 adults and 2 of the young monkeys with deciduous dentitions. The caries distribution is shown in Table I. Eighty-five of the 427 teeth examined were carious, 22 of which were permanent and 63 deciduous. Altogether 171 lesions were detected: 34 lesions in 22 permanent teeth and 137 lesions in 63 deciduous teeth. All of the lesions were located in pits and fissures in posterior teeth except 2 that were smooth surface caries involving the mesiolingual aspect of the maxillary deciduous canines in one specimen.

TABLE I

CARIES DISTRIBUTION IN THE TEETH OF THE CYNOMOLGUS MONKEY

	PERMANENT TEETH				DECIDUOUS TEETH			
	MAXILLARY		MANDIBULAR		MAXILLARY		MANDIBULAR	
	NO. EXAMINED	NO. CARIOUS	NO. EXAMINED	NO. CARIOUS	NO. EXAMINED	NO. CARIOUS	NO. EXAMINED	NO. CARIOUS
Incisors	20	0	20	0	48	0	46	0
Canines	8	0	8	0	28	2	27	0
Premolars	16	0	16	0	–	–	–	–
Molars	44	11	44	11	52	39	52	22
Total	86	11	88	11	128	41	125	22

It is interesting to note that only the very young or the older monkeys were caries-free while those of intermediate age had frequent carious lesions. All 3 adult monkeys maintained in our laboratory on a purified ration, where one would actually expect an increased caries experience, did not have any

Fig. 1.

Fig. 3.

Fig. 2.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 1.—Jaws of a young cynomolgus monkey with all decidous teeth and first permanent molars present. Note the typical occlusion, anterior edge-to-edge bite, Angle Class I molar relationship and the space between the lateral incisors and canines.

Fig. 2.—Radiograph of a young cynomolgus monkey with erupted first permanent molars. Note the position and degree of calcification of the unerupted permanent teeth; the high position and relatively little calcification of the canines; no detectable calcification of the third molars.

Fig. 3.—Jaws of an adult male cynomolgus monkey with all permanent teeth present. Note the well-developed tusklike canines and the peculiar form of the mandibular first premolar, with enamel buccally covering a long clinical crown. These are typical characteristics in the adult male.

Fig. 4.—Jaws of an adult female cynomolgus monkey. Note the difference in the size and shape of the canines when compared with the male in Fig. 3. Some calculus deposit and recession of the alveolar bone can be seen.

Figs. 5 and 6.—Radiographs of the cynomolgus monkey which was born and raised in our laboratory of parents maintained on a purified diet. Permanent canines and mandibular first premolars are beginning to erupt and calcification of the third molars has started. Note the asymmetry of the maxillae, which was probably conducive to the orthodontic discrepancies in this animal.

lesions. However, these animals had been captured and brought into captivity as adults with all permanent teeth erupted. Caries development may be enhanced in this species, if the environment of captivity is initiated prior to the completion of tooth formation. Possibly the 2 young caries-free monkeys that had only deciduous teeth might have gone on to develop a greater incidence of carious lesions if they had remained for an appreciable period in captivity.

The steepness of the cusps of the newly erupted molars, the cuspal inclined planes often joining at a less than 90-degree angle, might also be one of the contributing factors to caries initiation because food debris would have a greater tendency to remain in such deep and narrow developmental grooves. Since the histologic sections of numerous specimens revealed either no enamel or only a thin enamel bridge at the fusion point of developmental lobes, this situation may have contributed to the ease of caries initiation.

Aside from these defective fissures, no other morphologic abnormalities such as mottling, enamel hypoplasia, or interglobular dentin, were seen.

SUMMARY

The dentition of 17 cynomolgus monkeys was studied. Only 5 of the animals examined were caries-free—either the very young ones or those which were captured when adult, with all permanent teeth erupted.

This species has quite steeply cusped posterior teeth, sometimes with lack of enamel at the fusion points of developmental lobes. These factors might facilitate the initiation of carious lesions.

Periodontal structures did not show any pathosis in 16 of the animals. Only one adult had calculus deposits and some alveolar bone recession.

Orthodontic discrepancies were observed in 2 of the animals examined.

This species may become of major importance in dental research due to the fairly high susceptibility to dental caries. In addition, this species is appreciably smaller than the common rhesus monkey and breeds reasonably well in the laboratory when maintained on purified diets.

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THE EFFECT OF PRESSURE ON THE HEALING OF THE EXPOSED HUMAN DENTAL PULP

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IT is generally assumed that in applying drugs to the exposed surface of the dental pulp, extreme care should be employed to prevent any pressure; otherwise, damage of tissue will result. This point of view was reported by Pfaff[1, 10] who was one of the first to try to maintain the vitality of an exposed pulp. In order to avoid pressure on the wound, he covered the pulp by means of gold plate. The filling material was subsequently placed over this.

Hermann's[3, 4] introduction of calcium hydroxide† started a new epoch in the treatment of the dental pulp. In spite of new methods for pulp treatment, the use of pressure upon the pulp remained a "paradoxical" or "heroic" method.

Since there is no clinical or experimental report which actually deals with the effect of pressure upon the dental pulp, the purpose of this work has been to study what changes occur in the exposed pulp as a result of pressure and in which way the pressure effects the healing of the pulpal wound.

MATERIAL AND METHODS

Material for the investigation was 42 permanent (mostly premolars), noncarious or slightly carious teeth of school children and adults, which were to be extracted for orthodontic or prosthetic reasons. For purpose of control, 6 untreated and noncarious teeth, on which no pulpal pressure was used, taken from the same patients, were also examined.

Generally, occlusal cavities were prepared. All dentin fragments and chips resulting from cavity preparation were washed out as far as possible by means of a water spray. After application of 10 per cent iodine solution to the cavity, the pulp was exposed. Before the paste was applied, the opening in the chamber roof was sufficiently enlarged to permit a more complete elimination of dentin chips during pulpal bleeding. Finally, the pulp wound

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†Excellent reviews on the treatment of the exposed dental pulp with calcium hydroxide are to be found in References 1 and 8.

TABLE I

THE EFFECT OF PRESSURE ON THE HEALING OF EXPOSED PULPS IN 40 TEETH

CASE NO.	AGE OF PATIENT	TOOTH	TEMPORARY DISCOMFORT	DURATION OF THE POST-OPERATIVE PERIOD	DEGREE OF COMPACTION	TYPE OF HEALING	INFLAM-MATION
368	12	/ 5		0d	to		
353	38	/ 8		0d	cr		
357	13	/ 6	0	1d	cr	dc	
319	12	4 /	0	2d	cr	dc	li
321	10	/ 4	0	2d	to	n	p
333	8	4 /	0	3d	to	dc	
338	14	/ 2	0	3d	to	dc	
359	15	5 /	0	3d	cr	ft	
356	13	6 /	0	3d	cr	dc	li
352	40	8 /	0	5d	cr	dc	li
358	15	5 /	0	5d	cr	n	p
369	14	4 /	0	5d	to	br	
322	10	4 /	0	5d	to	dc	
337	14	/ 4	0	6d	cr	ft	p
340	14	/ 4	+	6d	cr	br	
372	13	4 /	0	6d	to	dc	
355	12	/ 4	0	7d	to	ft	
365	35	/ 8	0	7d	to	ft	
320	42	3 /	0	9d	to	ft	p
370	10	4 /	0	13d	to	br	
350	14	2 /	+	2w	cr	n	p
317	12	/ 4	0	2w	cr	br	
366	12	/ 4	–	1m	cr	ft	
307	9	/ 4	0	1m	to	dc	li
330	24	/ 8	0	1¼m	cr	n	p
348	10	/ 4	0	1¼m	cr	n	p
331	23	8 /	0	1½m	cr	br	li
334	12	/ 4	0	1¾m	cr	br	li
308	15	/ 4	0	2m	to	br	
362	14	4 /	0	2m	to	n	p
310	12	/ 4	+	2¼m	to	dc	
354	28	/ 8	0	2½m	cr	br	
302	12	5 /	0	5m	to	dc	li
325	14	5 /	0	5m	to	br	
312	12	5 /	+	6m	to	br	li
318	12	4 /	0	8½m	to	br	
304	13	/ 2	0	10m	cr	br	
303	13	4 /	–	13m	to	ft	
115	12	6 /	0	13m	cr	ft	
309	12	/ 4	0	14m	to	br	

(For Key to Abbreviations see opposite page.)

was cleaned and the bleeding was stopped by the use of 3 per cent hydrogen peroxide solution. Compacting of the pulp was accomplished by means of a thick paste pressed into the pulp chamber with a cotton pellet. In this manner, partial or complete compacting of the coronal pulp was obtained.

The paste used was a commercial preparation composed chiefly of calcium hydroxide in Ringer's solution. In order to thicken the paste and to reduce its corrosive effect, microcrystalline sulfathiazole was added to make up 60 to 80 per cent of the total. Due to the small amount of sulfathiazole actually used, no therapeutic action could be expected from it. Also, with a few exceptions, this paste contained an x-ray contrast medium, consisting of 1 to 2 per cent strontium salts. This combination will hereafter be referred to as CaS paste. Since this paste, once placed, did not become dislodged, an intermediate lining was unnecessary, and the cavities were subsequently sealed with amalgam or other filling material. The teeth were radiographed immediately after the operation and at the end of the observation period which ranged from 1 day to 14 months (Table I). In order to examine the condition of the pulp immediately following the use of pressure, 2 teeth were extracted ½ and 2 hours after the operation. Several days after the operation and at later periods, the teeth were examined for pain symptoms upon application of heat, cold, or percussion.

The extracted teeth were fixed in a 10 per cent formalin solution immediately after extraction. The long period necessary for decalcification of teeth during histologic preparation may be one of the reasons for the presence of the artefacts seen in the pulpal sections.[5, 6, 11] In order to minimize artefact, the decalcification time was reduced in many of the teeth by grinding off the bulk of the dentin. Decalcification was effected in a 5 per cent solution of trichloracetic acid and paraffin was used for embedding. All the teeth were sectioned in the longitudinal plane. The sections were stained with hematoxylin and eosin, azan stain, or with Bock, Hansen, Mallory, hematoxylin-ponceau-fuchsin, and Bielschowsky stains.

OBSERVATIONS

Clinical examination within the first days after the operation showed that in spite of strong pressure on the pulp, only 1 in 9 subjects experienced

Key to Abbreviations

0 = Asymptomatic case.
\+ = Presence of discomfort.
\- = Patient did not return for observation.
d = Days.
w = Weeks.
m = Months.
cr = Partial compacting of the coronal pulp.
to = Complete compacting of the coronal pulp.
dc = Only dysfunctional zone is present.
ft = Beginning formation of bridge in form of so-called fibrillar dentin.
br = Bridge of fibrillar, irregular, or regular dentin (with or without previous formation of the dysfunctional zone).
n = Absence of healing, or incomplete healing (in the case of the radicular pulp in premolars or molars, n is shown even if one of the roots shows healing).
ll = Small round-cell infiltration.
p = Abscess; or diffuse or circumscribed, chronic or subacute pulpitis (in the case of radicular pulps of premolars or molars even if only one pulp shows inflammation).

minor discomfort of short duration from percussion or upon application of cold. One tooth was extracted because of acute pulpitis.* The remaining teeth, with the exception of Case 350, were asymptomatic throughout the observation period. X-ray examination did not reveal any loss of periapical bone.

Histologic findings in the teeth that had been extracted immediately or a few days after treatment showed that the pressure affects only the superficial portion of the pulp (Figs. 1, 3). Where the opening of the pulpal roof was small and only slight pressure was applied or the paste was not pushed too deeply, only a localized compacting occurred within the coronal pulp (partial compacting of the coronal pulp) (Figs. 1, 3, 5, 8). The compacted tissue stroma appears as a more or less wide collagenous capsule which demarcates the remainder of the pulp from the CaS paste (Figs. 2, 4, 6, 11). When the paste is pressed deeper into the coronal pulp (complete compacting of the coronal pulp) (Fig. 13), the big bulk of the compacted tissue sometimes suggests a fibrous metaplasia of the pulp (Fig. 10). The compacted tissue, after various periods of time sometimes shows hyaline degeneration (Fig. 14). The remaining pulp was unaffected by the pressure (Figs. 2, 4). A striking feature here was the insignificance or the absence of postoperative hemorrhages and hematomas. It was a noteworthy fact that an initial necrosis of the surface of the lesion which usually develops as the result of application of

*This tooth was not examined histologically.

Fig. 1.—Compaction of the coronal pulp 2 hours after operation. The space (in the chamber of the crown) which was formerly occupied by the pulp contains remnants of the CaS paste left during histologic preparation. The pulp tissue which formerly occupied the chamber of the crown became compacted and now shows a thick fibrous capsule (*X*) which separates the underlying pulp from the paste. There are no hemorrhages below or above the fibrous capsule. (/8, Case No. 353, azan stain; Orig. Mag. ×10.)

Fig. 2.—Portion of compacted pulp from Fig. 1. The previously fine network of fibers of area beneath exposure now appears as dense fibrous capsule (*X*). In capsule, compacted pulp cells are spindle-shaped. Below capsule pulp tissue appears normal, and blood vessels (*b*) show normal-sized lumen. Above capsule are the remnants of CaS paste. (Hematoxylin and eosin stain; Orig. Mag. ×345.)

Fig. 3.—Normal pulp below fibrous capsule 1 day after partial compaction of coronal pulp. No hematoma nor hemorrhage is present below or above fibrous capsule (*X*). (/6, Case No. 357, hematoxylin and eosin stain; Orig. Mag. ×10.)

Fig. 4.—An area of compacted zone from Fig. 3. Below fibrous capsule (*X*), pulp tissue is normal in appearance except for slight hyperemia. Note absence of necrotic tissue and hemorrhage. *CaS* = space formerly occupied by the paste. (Hematoxylin and eosin stain; Orig. Mag. ×130.)

Fig. 5.—In spite of partial compaction of coronal pulp and a short postoperative period of only 6 days, dentinal bridge (*br*) developed. No dysfunctional zone is present, and remnants of CaS paste are separated from bridge be means of an artificial space (*sp*) caused by shrinkage during histologic preparation. Disruption of pulpal tissue below the bridge is also due to artefact. (/4, Case No. 340, Bock's stain; Orig. Mag. ×6.)

Fig. 6.—Remnants of CaS paste above artificial space seen in Fig. 5. *CaS* = remnants of the paste. *X* = the compacted pulp tissue in the form of fibrous capsule. The compacted pulpal cells of the capsule appear spindle-shaped. (Hematoxylin and eosin stain; Orig. Mag. ×45.)

Fig. 7.—Higher magnification of the bridge seen in Fig. 5, demonstrating its fibrillary structure, which suggests a so-called "primitive dentin." (Bock's stain; Orig. Mag. ×120.)

Fig. 8.—Formation of dentinal bridges following a partial compacting of pulp 2½ months postoperatively. There is no intermediate zone of dysfunctional pulpal tissue between fibrous capsule (*X*) and bridge. (/8, Case No. 354, hematoxylin and eosin stain; Orig. Mag. ×15.)

Fig. 9.—Bridge area seen below pulpal roof circled in Fig. 8. This bridge has osteodentin structure. The pulp tissue shows an artificial reticulation and vacuolization of odontoblasts. (Hematoxylin and eosin stain; Orig. Mag. ×135.)

(For legends see opposite page.)

calcium hydroxide (which is a strong basic drug[1, 8]) was not pronounced, or in the majority of cases it was absent (Figs. 4, 7, 8, 10). More or less large necrotic areas of tissues were observed in unsuccessful cases with suppurated pulps, and, also, in one tooth where the paste had been pressed through the apical foramen with a plugger.*

In spite of exertion of pressure on the pulp, regeneration of the dentinal wall generally occurred. In some pulps, the formation of the dentinal bridge took place directly below the fibrous capsule (Figs. 5, 8, 10), within 5 to 6 days postoperatively (Fig. 5). In other pulps a dysfunctional zone first formed beneath the fibrous capsule, and the dentinal bridge became established apically to this area later (Figs. 11, 13, 14). The dysfunctional zone showed various characteristics. In some cases, obvious atrophy of pulp tissue was seen (Fig. 13). In such pulps, dystrophic calcifications were present, in contrast to the absence or the presence of only insignificant calcification in cases where bridges developed directly below the fibrous capsule. Also, the old odontoblasts adjacent to the dentin wall in this zone showed no capacity for the deposition of new layer of dentin (Fig. 14). In other pulps, the dysfunctional zone showed a more or less pronounced fibrous structure. In a few cases, development of collagenous fibers was so marked that it suggested the structure of scar tissue (Fig. 12). In some cases, the healing of the pulpal wound was limited to the formation of this dysfunctional zone.

Dentinal bridges developed first as irregular dentin of varied appearance, such as fibrillar dentin (Fig. 7), osteocementum, osteodentin (Fig. 9), and cementodentin (Fig. 10) which became later supplemented by a layer of more regular dentin (Figs. 11, 14). There was no direct relationship between either the structural features of the dentinal bridge or the occurrence of a dysfunctional zone and the depth to which initial pressure had been

*This tooth is not included in the table.

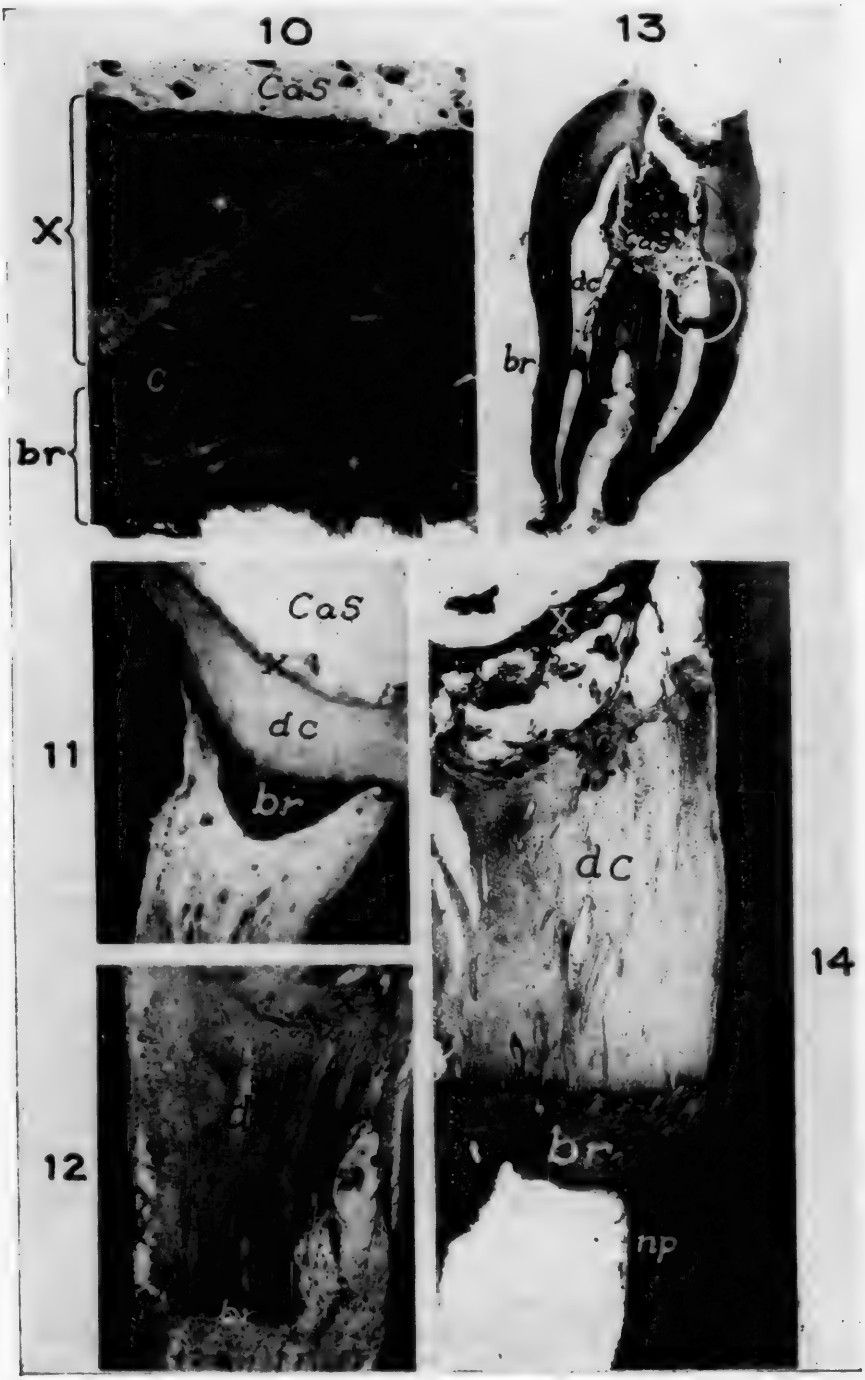
Fig. 10.—Compaction of entire coronal pulp produced thick bulk of fibrous tissue, which suggests fibrous metaplasia of pulp 13 days postoperatively. Below compacted pulp (*X*), a bridge (*br*) of cementodentin structure developed without previous formation of an intermediate dysfunctional zone. *CaS* = remnants of the paste. *C* = dystrophic calcification. (4/. Case No. 370, Bock's stain; Orig. Mag. ×135.)

Fig. 11.—Results of pulpal compaction 8½ months postoperatively. *X* = fibrous capsule. *dc* = intermediate dysfunctional zone which was formed in the first stage of healing. *br* = dentinal bridge with regular structure. *CaS* = space formerly occupied by the paste. (4/. Case No. 318, Hansen's stain; Orig. Mag. ×44.)

Fig. 12.—Dense collagenous tissue, suggestive of scar tissue, present in dysfunctional pulpal zone (*dc*), 2¼ months postoperatively. There is a rudimentary formation of a dentinal bridge (*br*). *X* = fibrous capsule. (/4, Case No. 310, hematoxylin and eosin stain; Orig. Mag. ×100.)

Fig. 13.—Dentinal bridges developed in radicular pulp of each root in spite of complete compaction of the coronal pulp 14 months postoperatively. Pulp chamber is filled with remnants of CaS paste. Because of pulpal dysfunction (*dc*) during first stage of healing, bridges (*br*) were formed apically far below the level of bifurcation. (/4, Case No. 309, hematoxylin and eosin stain; Orig. Mag. ×4.)

Fig. 14.—Pulp of root (circled) on right side of Fig. 13 in higher magnification. Zone of dysfunction shows atrophy of pulpal tissue. Odontoblasts of this zone are still present, but subsequent to operation they ceased producing dentin. Axial odontoblasts below the bridge (*br*) produced a new layer of dentin (*np*). *X* = degenerated fibrous capsule. *c* = layer of dystrophic calcification. The pulp below the bridge was lost in this section during histologic preparation. (Hematoxylin and eosin stain; Orig. Mag. ×40.)



(For legends see opposite page.)

exerted. In some cases, whether formed in the crown or in the root, the dentinal bridges remained in the stage of formation of a fibrillar dentin, irrespective of the long postoperative period (Table I).

Inflammation of the pulp was present in 16 teeth. In 8 pulps, small areas of round-cell infiltration were localized either above (in the dysfunctional zone) or below the bridge. It may be that the number of these small inflammatory foci was larger, since artefact might sometimes preclude locating them. A low intensity of inflammatory reaction did not affect regenerative capacity of the pulp. In 8 remaining cases, a more advanced chronic or subacute pulpitis was present, revealing a diffuse or circumscribed infiltration with lymphocytes, plasma cells or polymorphonuclear leukocytes or abscesses. In these teeth, the formation of bridges was disturbed or completely absent. It is noteworthy that in 6 teeth of this group a few or many dentin fragments were present at the wound site or numerous small dentin chips were disseminated deeper in the pulp. As a rule, the apical portion of the pulp was free of inflammation.

Some healthy and inflamed pulps showed unavoidable artefacts such as "reticulation," "odontoblastic vacuolization," "edema," and "cyst formation." The same alterations could be seen in control teeth.

DISCUSSION

The clinical results and histologic findings reveal that the opinion that pressure must not be applied to the exposed pulpal surface for fear that the tissue might be destroyed does not hold true. The results show that the pressure affects only the superficial portion of the pulp and that regeneration of the dentin wall in form of a bridge can even develop directly below the compacted portion of the pulp. The effect obtained may be explained by the presence of collagenous fibers throughout the pulpal tissue. At the pulpal wall many of the fibers continue into the dentin. When pressure is applied, these fibers become compacted directly in advance of the instrument employed. The width of the fibrous capsule increases in proportion to the extent of the pressure. Laterally to the point of pressure, the fiber attachment to the dentinal wall is broken to an extent related to the intensity of the pressure. The effect of the pressure is limited to the fibrous capsule since the remaining fibrous network attaches the pulp to the dentin wall and does not allow extension of the pressure effect to great depth. This also explains why the majority of subjects were asymptomatic subsequent to the operation.

In some cases, the pulp below the fibrous capsule showed atrophy or fibrous degeneration. A dentin bridge became established apically to this dysfunctional zone. That this feature is not related to the use of pressure is demonstrated through similar results in pulpal healing obtained by other investigators[1] who employed calcium hydroxide without pressure.

In one fifth of the operated pulps, results were unsuccessful due to suppuration. Since the majority of inflamed pulps showed the presence of dentin chips or fragments, it appears evident that the inflammatory reaction resulting in

suppuration may have been caused by these dentin chips and not by pressure. Also, in pulps without inflammation, dentin fragments disturbed the healing[7] and interfered with formation of dentin bridges having a regular structure. In 2 cases, infection was provoked because the amalgam fillings were lost or carious dentin was not completely removed during the operation.

The last question remains whether pressure at the exposed surface of the pulp has a beneficial effect on healing. It is well established in surgery that the formation of a postoperative hematoma within a wound prevents coaptation of the wound margins, prolongs cicatrication, and predisposes to infection.[9] Presumably, the effect of postoperative hemorrhage or of a hematoma on the healing of a pulpal wound[2] would not be an exception. In the case of pulp capping or pulpotomy, where drugs are applied without pressure, the operative hemorrhages are usually treated with solutions of 3 per cent H_2O_2, $Ca(OH)_2$ or physiologic saline. Nevertheless, that this treatment of hemorrhages is not adequate and that a postoperative hematoma develops under the dressing can be seen in the illustrations of other workers[1] where an "organized blood clot" is even presented as the regular feature in the healing of the pulpal wound.

In our case of pulpal treatment, postoperative hematoma or hemorrhage generally did not develop because, through the application of pressure, the exposed and traumatized blood vessels become compressed, and bleeding stops automatically and permanently in the course of the operation. That pressure had a beneficial effect on healing, brought about by the prevention of bleeding, is demonstrated by the finding that, in spite of wide exposure, bridges can develop directly below the compacted portion of the pulp.

SUMMARY AND CONCLUSIONS

1. The effect of pressure, obtained by means of a thick paste (composed of calcium hydroxide, sulfathiazole, and strontium salts), on exposed, healthy pulp tissue was studied in 48 permanent teeth.

2. Teeth were asymptomatic in the majority of cases and only slight discomfort of short duration was evident in a minority of cases when pressure was exerted on the pulp.

3. As observed histologically, the effects of pressure were the compacting of the connective tissue stroma in the superficial portion of pulp, which appeared as a fibrous capsule or as a fibrous metaplasia. Below this capsule, the tissue remained undisturbed.

4. Restoration of a dentinal wall developed in 2 ways; (a) the formation of a bridge took place directly below the compacted portion of the pulp, and (b) establishment of a dentinal bridge was preceded by formation of a dysfunctional zone of pulpal tissue.

5. The application of pressure favored the healing of the pulpal wound since it controlled postoperative bleeding.

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EFFECTS OF DENTAL DRUGS AND MATERIALS ON THE RATE OF OXYGEN CONSUMPTION IN BOVINE DENTAL PULP

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THE purpose of this study was to ascertain what influence various drugs, pulp capping, and restorative materials, commonly used in dental practice, might have upon respiration in pulp tissue. Interest in this subject was stimulated by numerous and sometimes conflicting speculations on the mode of action of these materials, and by occasional diverse recommendations for their clinical application.

The rate of endogenous oxygen consumption in normal bovine dental pulp was determined by Flieder and Fisher[1] to be 0.56 ml. per hour per milligram of dried tissue weight. Subsequent studies by Fisher, Schumacher, Robinson, Albers, and Sharbondy[2] indicated that close approximations of this value could be achieved only by using high concentrations of oxygen despite the apparently low level of respiratory activity of this tissue. The combined data of these 2 investigations are the bases for regarding the mean normal oxygen quotient of bovine dental pulp to be 0.55 (S.D. ±0.14) for purposes of comparison in the present study.

It is assumed that the rate of oxygen utilization by a tissue is an approximate indication of the level of its metabolic activity. Hence, significant deviations from the normal rate of oxygen consumption by the dental pulp under the influence of exogenous materials should yield some information on the physiologic aspects of these relationships. This study is exploratory in nature and serves to point out the direction of possible further investigations of more specific character.

METHOD AND RESULTS

The manometric technic employed in this investigation was the direct method of Warburg as described by Umbreit, Burris, and Stauffer.[3] The gas volume of the flask-manometer system was determined by the method of Lazarow.[4] The pulp tissue was obtained from the teeth of young cattle immediately after slaughter at a local abbatoir. It was placed immediately in chilled Krebs-Ringer's solution and transported to the laboratory without delay. The individual pulps were sliced with a Stadie-Riggs slicer to less than 1 mm. in thickness. The suspending solution in the Warburg vessels was Krebs-Ringer-phosphate solution with the buffer adjusted to a pH of 7.4. The

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approximate weight of the average dried tissue sample from most of the determinations was 20 mg. The suspending solution was oxygenated prior to the addition of the tissue slices and the flask-manometer system was flushed with oxygen for several minutes to insure that the oxygen in the gas phase was close to 1 atmosphere of pressure.

In a series of 20 determinations, 2 BB-sized pellets of zinc oxide-eugenol paste were added to the suspending fluid prior to the addition of the tissue slices. The pellets had been prepared 18 hours before they were used to allow ample time for setting in order to avoid disintegration in the flask. In a second series of 21 determinations, a saturated solution of eugenol in water was used in the preparation of the Krebs-Ringer-phosphate solution. Eugenol is very slightly soluble in water.[5] A saturated solution of calcium hydroxide was used in the preparation of the Krebs-Ringer-phosphate solution for a third series of 21 oxygen quotient determinations. The solubility of calcium hydroxide in water is 0.185 Gm. per 100 ml. at 0° C. In 22 determinations, 2 silver amalgam pellets, aggregating approximately 0.33 Gm., were added to each flask of tissue. The silver amalgam was prepared in pellet form about 1 hour prior to use. A drop of mercury about the size of a BB shot was added to each flask in 30 determinations. In 11 samples, 2 pellets of zinc phosphate cement were added to each flask. The cement pellets of BB size were made from a thick mixture prepared just prior to use. In 13 determinations, 1 drop of a 1:1000 Adrenalin hydrochloride solution was added to each milliliter of the suspending solution. In 15 determinations of the QO_2 the Krebs-Ringer-phosphate solution was used to prepare a 2 per cent procaine hydrochloride solution. This, in turn, served as the suspending solution for the tissue slices.

The results of all determinations are listed in Table I.

TABLE I
EFFECTS OF DENTAL MATERIALS ON QO_2 OF BOVINE DENTAL PULP

MATERIAL	NUMBER OF DETERMINATIONS	QO_2	S.D.	RANGE
—	78	0.55	0.14	0.26-0.95
ZnO-eugenol	20	0.42	0.22	0.07-0.87
Eugenol	21	0.31	0.13	0.09-0.48
Ca OH	21	0.42	0.24	0.10-1.09
Ag amalgam	22	0.33	0.08	0.10-0.47
Mercury	30	0.33	0.21	0.06-0.92
Zn phosphate cement	11	0.32	0.18	0.14-0.72
Adrenalin	13	0.34	0.24	0.05-0.79
Procaine	15	0.16	0.06	0.09-0.33

DISCUSSION

The data in Table I suggest that all of the materials tested have a depressant effect upon the respiration of bovine dental pulp. The range of values in the individual series and the magnitude of the standard deviation of the mean QO_2 in several instances may raise some doubt as to the significance of certain of these mean values. This matter was of concern to the present investigators and pertinent studies now in progress indicate the probability of improvement in this situation by modification of currently accepted technics. The

recommended practice of correcting the reading of each manometer of the Warburg apparatus by the reading of a single blank flask-manometer system known as the thermobarometer has been found to be inadequate when working with tissues of such low respiratory activity as the dental pulp. It has been observed that the standard deviation of the mean QO_2 of a series of determinations made at the same time and during a period of barometric stability tends to be much smaller than under more varied circumstances. Consequently, it is believed there is justification for accepting the QO_2 values as indicative of general effects that require more extensive measurement before more precise values can be established.

The most pronounced depression of the QO_2 was observed in connection with procaine hydrochloride. If it is assumed that the action of procaine upon the respiratory mechanism of pulp tissue is generally the same as that of cocaine and certain barbiturates on brain and kidney tissue, our observations on the dental pulp are in agreement with work reported by Quastel,[6] and by Yamamoto and Kuragochi,[7] who observed considerable reduction in the QO_2 of brain tissue when working with barbiturates and cocaine, respectively. Yamamoto and Kuragochi observed a similar effect in cocainized kidney tissue. Quastel's work on nervous tissue indicated that the effect was upon the cytochrome-flavo-protein-coenzyme segment of the respiratory enzyme system.

Fusco and Guarino[8] found that mercury had an inhibitory effect on succinic dehydrogenase in concentrations as low as 0.0001 N solutions. Hirade and Hayasi[9] observed that the succinoxidase activity of rat liver mitochondria declined under the influence of increasing concentrations of copper and mercury ions. Potter and DuBois[10] also found that copper and zinc ions had an inhibitory effect on succinic dehydrogenase activity. These observations suggest an explanation of the behavior of pulp tissue respiration as measured in the present study in connection with mercury. The similarity of the mean QO_2 obtained with mercury and with freshly prepared silver amalgam may indicate that mercury is the influential factor in both cases.

No explanation is offered with respect to the action eugenol and calcium hydroxide have in depressing the oxygen quotient other than that provided by the well-known histologic changes in pulp tissue produced by these drugs. It is believed that the less drastic effect produced by hardened zinc-oxide eugenol paste is merely an expression of the lesser availability of eugenol in this state.

The influence of freshly prepared zinc phosphate cement in reducing the QO_2 possibly is due to a complex of factors. The most readily apparent of these is the acidification of the suspending solution through exhaustion of its buffer by reaction with the still free orthophosphoric acid in the cement mixture. When depression of the QO_2 by zinc phosphate cement was observed, pH measurements were made on similar quantities of suspending solution in which only cement pellets were immersed. At the end of the first 5 minutes the solution had dropped from pH 7.11 to pH 6.89, and to pH 6.73 at the end of 1 hour.

The reason for the reduced QO_2 in relation to Adrenalin is not explained.

SUMMARY

This preliminary study of the effects of several drugs and materials used in dental practice on the oxygen consumption in bovine dental pulp indicates that several of them probably have a significant inhibitory effect on respiratory activity in this tissue. Further investigations are necessary to reveal the mechanisms by which these inhibitions are accomplished.

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OBSERVATIONS IN VARIATION IN X-RAY DENSITY WITH BONE LOSS IN THE HUMAN MANDIBLE

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THE dental radiograph is necessary for adequate diagnosis and prognosis of periodontal problems. Clinical experience indicates that it has some shortcomings. The dental radiograph frequently appears to form an incomplete basis for the premature loss of periodontally involved teeth. With this in mind an investigation was planned to explore the limitations of the dental radiograph as an adjunct in diagnosis and prognosis in periodontal disease.

A macerated human mandible was sectioned and 3 segments were selected for the experiments. Each segment had 1 or 2 teeth in situ in the alveolus. The alveolar process was divided into areas of investigation: (a) the interradicular bone, lamina dura and crest identified as alveolar bone proper, (b) trabecular bone away from the root end identified as supporting bone, and (c) the cancellous bone near the root end but coronal to it, identified as trabecular bone.

The specimens were demineralized in a 10 per cent solution of nitric acid solution and radiographs were taken. An electronic densitometer was used to record the density changes in the radiographs. Thus, a relationship was established between exposed film, demineralized bone, and the density change of the specimen.

In clinical x-ray reading the measurement of radiographic changes is commonly made with the human eye. In these experiments an electronic transmission densitometer was substituted for the human eye. The response of this instrument, when measuring light transmitted through a radiograph, can be indexed to react in a manner similar to the light intensity response of the human eye. It is not intended that an electronic transmission densitometer be used as a routine diagnostic tool. The instrument is used to approximate the eye without the weaknesses of fatigue and variable resolution to which the eye is subject and to standardize results.

Reliability and constancy of reading with changing ambient conditions are additional reasons for the instrument reading as the response of a standardized and reliable human eye. Thus it is possible to obtain a standardized record of x-ray density with various degrees of demineralization of bone so that proper evaluation can be made of how this response varies according to the following factors:

1. Knowledge of the optical density of a radiograph of nondemineralized bone tissue.

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2. Variation of optical density with degree of demineralization of bone tissue.

3. Variation of optical density with changes in: (a) incident x-ray intensity, (b) sensitivity variations from batch to batch of x-ray films, (c) sensitivity variations from area to area on a single x-ray film, (d) exposure time, and (e) developing solutions and time.

4. Knowledge of the varying sensitivity of the human eye with ambient light intensity.

METHOD AND MATERIALS

A set of experiments were designed so that 3a, 3b, 3c, 3d, 3e and 4 caused either little or no effect so that changes due to factors 1 and 2 might be measured. The factors affecting the results were controlled in the following ways. For 3a, tests were made with the Ritter Dual X x-ray unit set at a fixed value of operating voltage and current with the tube to film distance constant; for 3b, tests were made from the same batch of Eastman Kodak ultra speed intra-oral films indicated by the batch number which was the same for all the films used; for 3c, variations of photometric readings for a blank, exposed area on the developed film gave the indications of such variations; for 3d, the exposure time was the same for all the tests; and for 3e, the developing time and solutions used were standardized.

Three sections of a specimen taken from a human mandible were used in these experiments. The sections were x-rayed at various time intervals during the demineralization process, after being washed and dried under vacuum. The controlled parameters were current, 13 ma.; voltage, 63 kv.; exposure time, 3 seconds; developing time at 68° F., 4 minutes; fixing time, 15 minutes; and washing time (in running water), 30 minutes.

The same specimens were also radiographed with the demineralization time constant and the above parameters varied, i.e., kv., 65 and 70; ma., 10 and 15; exposure times varied and were repeated at 2, 2½, 3 and 3½ seconds; and developing, fixing, and washing times were constant.

Photometric measurements were made of only those samples described above as the group taken at 13 ma., 63 kv., and exposure time 3 seconds. Each radiograph was indexed and examined with the densitometer in the following areas: (1) developed film background, (2) supporting bone tissue, (3) trabecular bone (interseptal), and (4) alveolar bone proper. The response of the densitometer to an incident transmission of light intensity was indicated by a meter reading.

If the initial intensity of x-ray on the film is designated as P_o, and the light transmitted through the film at P_t, then the transmission is defined as:

$$\text{*Transmission in per cent} = 100 \times P_t/P_o \qquad (1)$$

The optical density is defined as:

$$\text{†Density} = -\log_{10}(P_t/P_o) \qquad (2)$$

*The definition is described in *Manual for Electronic Transmission Photometer*, Photovolt Corp., page 256.

†Expression of this definition in terms of logarithmic units, is given in *Handbook of Physics and Chemistry* under definition of decibels, U. S. Rubber Publishing Co., 1955.

The response of the eye can in many ways be considered the negative of the above quantity[3]:

$$\text{Response of the eye} = -\text{Density} = \text{Log}_{10}\ (P_t/P_o) \quad (3)$$

The response of the eye here is to visible light which is not directly proportional to the light that falls on it. If it could be measured in arbitrary units it would vary directly with multiples of the incident light. Table I illustrates this point.

TABLE I

RESPONSE OF EYE IN ARBITRARY UNITS	INCIDENT LIGHT IN ARBITRARY UNITS
1	1
2	2
3	4
4	8

The response of the densitometer is measured in units of density and, therefore, in units similar to the eye's response. Also, when the human eye looks at an x-ray film it notes the background area density since it is concerned with variations in density from this background.

The x-ray absorption formula states:

$$P_t' = P_o' e^{-\alpha x} \quad (4)$$

Where P_o' = x-ray intensity incident on bone specimen.
P_t' = x-ray intensity emerging from bone specimen.
x = Thickness of bone specimen.
α = Attenuation constant which is a function of the material which the x-rays traverse.

The variation of x-ray intensity with thickness of bone structure might be adequately described by the above relationship provided the bone is uniform in composition and the means of focusing are standardized.

It is interesting to note that when equation (4) is rewritten

$$P_t'/P_t' = e^{-\alpha x} \quad (5)$$

and taking the log of both sides and rearranging:

$$-\alpha x = \log_e\ (P_t'/P_o') \quad (6)$$

$$x = \frac{-2.3}{\alpha} \log_{10}\ (P_t'/P_o') \quad (7)$$

or the thickness of the bone specimen varies with the incident and transmitted x-ray intensity as does the response of the eye (as shown in equation 3) with incident and transmitted light intensity. Therefore, demonstrated is a relationship between response of the eye to the thickness of the bone specimen as determined by x-ray absorption, namely, response of eye $= -\left[\frac{\alpha}{2.3}\right] x$ (x = thickness).

For the purpose of estimating the eye's opinion of a bone area, the photometer reading for a bone area on the x-ray film was subtracted from the densitometer reading for the optical density of the background of the x-ray. This new quantity is called the relative density of the x-ray film and represents a quantity independent of the ambient shade of any film giving a number that should be proportional to the response of the human eye.

Plots were made of the per cent of bone loss vs. relative density of the x-ray film for the supporting, trabecular, and alveolar bone areas examined. The experiments (Nos. 1, 2, and 3) cover 3 specimens.

A plot was also made for experiment No. 2 of the per cent of bone loss vs. relative density for all of the bone areas examined in this experiment. This was done for the purpose of estimating how much differential demineralization of the bone areas occurred in nitric acid solution.

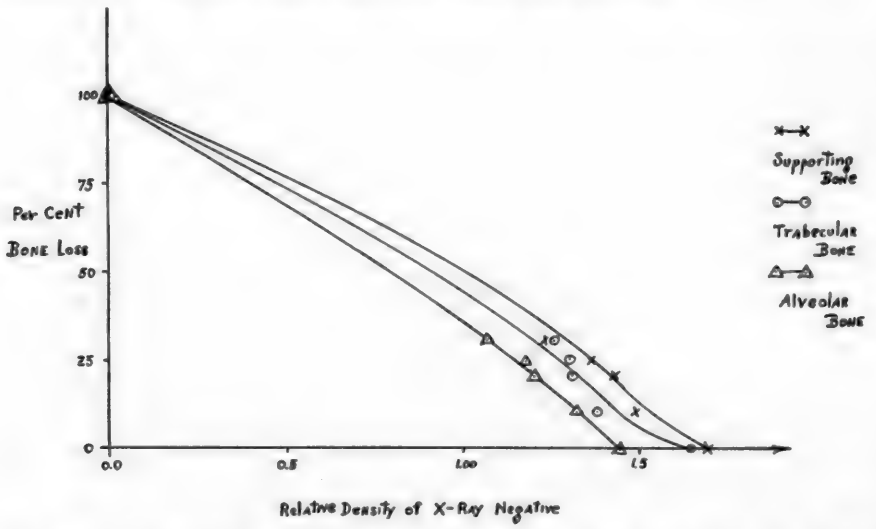

Fig. 1.—Experiment 2: per cent of bone loss vs. relative density of x-ray negative. This graph shows that the judgment of bone loss from the examination of an x-ray negative must vary with the type of bone being examined, i.e., a relative density of 1.0 may correspond to a bone loss of 35 per cent for alveolar bone and a bone loss of 50 per cent for supporting bone.

DISCUSSION

Fig. 1 shows the per cent of bone loss vs. relative density of the x-ray film for all of the bone areas of experiment No. 2. The fact that the slope and shape of the curves are very similar indicates that the demineralization of each of the different bone areas proceded at similar rates, and that therefore the nitric acid solutions dissolved all of the areas of the bone specimens uniformly.

Figs. 2, 3, and 4 indicate, for each of the bone areas, the per cent of bone loss vs. the relative density of the x-ray film. The amount of variation in bone loss for a specific area for the 3 experiments made can be determined as follows. Fig. 3 indicates that, for a relative density of 1.1, anywhere from 0 per cent to 30 per cent of the alveolar bone may be lost in the experiments

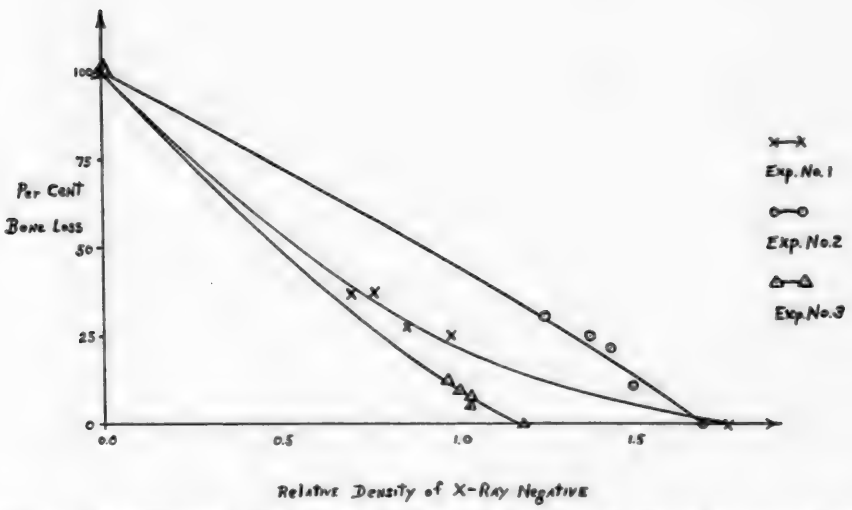

Fig. 2.—Supporting bone, per cent of bone loss vs. relative density of x-ray negatives. This graph shows that the same observed optical density on 3 different x-ray negatives corresponds to widely varying degrees of bone loss, i.e., a relative density of 1.0 corresponds to bone loss for supporting bone that varies from 9 to 43 per cent.

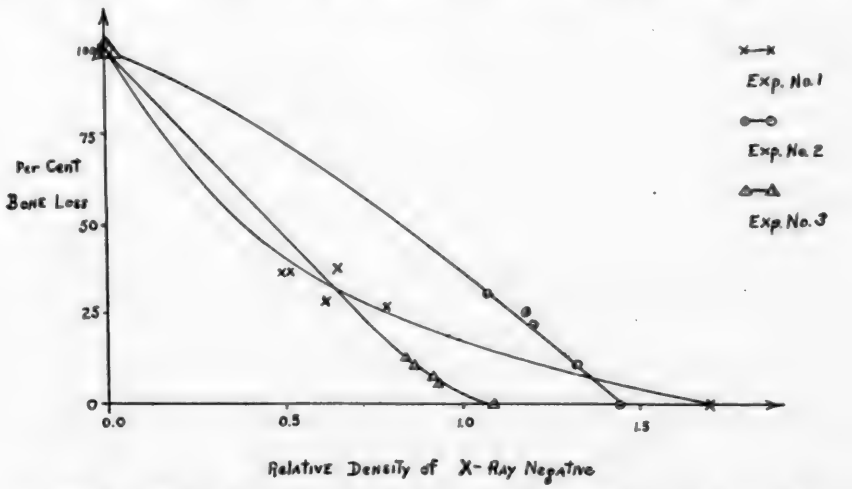

Fig. 3.—Alveolar bone, per cent of bone loss vs. relative density of x-ray negatives. This graph shows that the same observed optical density on 3 different x-ray negatives of alveolar bone corresponds to widely varying degrees of bone loss, i.e., a relative density of 1.0 corresponds to alveolar bone loss varying from 3 to 38 per cent.

that were conducted. This is the variation obtained with all the causes except knowledge of the optical density of a radiograph of nonmineralized bone and variation in optical density with degree of mineralization, either standardized or rigidly controlled. For the experiments conducted, the following maximum variations of percentage of bone loss were possible: supporting bone, 0 to 35 per cent, trabecular bone, 0 to 45 per cent, and alveolar bone, 0 to 30 per cent. It is observed then that with a given degree of bone loss there is a "wide" range of variation in x-ray density, even when a densitometer is used as the eye.

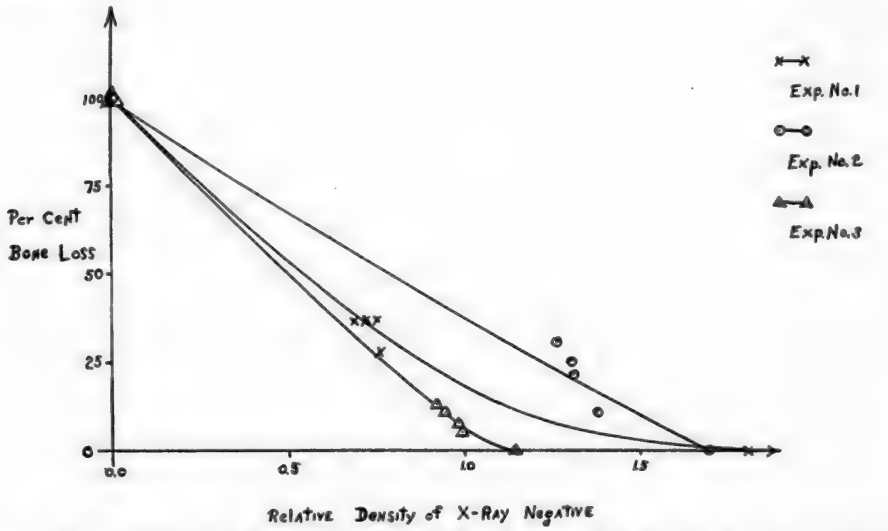

Fig. 4.—Trabecular bone, per cent of bone loss vs. relative density of x-ray negatives. This graph shows that the same observed optical density on 3 different x-ray negatives of trabecular bone corresponds to widely varying degrees of bone loss, i.e., a relative density of 1.0 corresponds to trabecular bone loss varying from 6 to 46 per cent.

This variation itself varies over the range of bone loss as can be observed in Figs. 2, 3, and 4, and also varies in the different bone areas within each individual bone specimen that was used in these experiments.

CONCLUSION

Since x-ray density is itself a variable with bone loss, even under partially controlled conditions, a clinical diagnosis and prognosis cannot be derived reliably by radiographic examination alone. Moreover, this would seem to be especially true for certain "degrees" of bone loss (where the variations are greatest), and for certain of the bony areas in the human mandible.

SUMMARY

1. A discussion of sources of error affecting the interpretation of the radiograph is presented.

2. Three experiments were undertaken to evaluate the contribution of some of the sources of error when others were controlled.

3. The uncontrolled sources of error are those which do not lend themselves to control in routine dental practice.

4. Results are presented which would seem to indicate that the variation of x-ray density with bone loss may influence diagnosis and prognosis in the practice of periodontics.

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INFLUENCE OF CERTAIN CONDENSATION PROCEDURES UPON THE MERCURY CONTENT OF AMALGAM RESTORATIONS

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THE clinical success of amalgam is influenced to a great extent by the final mercury content of the restoration. Various factors contribute to the residual mercury. One of these is the dryness of the individual increments which are used to build the restoration. There are different opinions regarding the amount of mercury desired in each increment. The most common method makes use of a somewhat wet initial portion followed by increments of increasing dryness.[1-3] In recent years some men have advocated the use of relatively dry increments throughout, supposedly in order to produce a more homogeneous restoration.[4-6] One investigation did evaluate 4 widely different procedures in regard to the mercury content of the individual increments and noted little difference in either the final mercury or the physical properties.[7] However, in that study a cylindrical specimen was used and the total mercury content in the entire mass was determined. It was felt that additional data would be desired to determine if some difference in mercury might not exist in different areas of a typical dental restoration, particularly the critical marginal areas, when these various technics were used. Likewise the effect of various mechanical condensers in eliminating residual mercury needs further study.

PROCEDURE

Over 100 restorations were condensed using the various technics to be described and the data presented are the mean for a minimum of 10 restorations with any given procedure. A standard class I preparation in a dentoform tooth was used throughout the study. Each restoration was carved to anatomic form and the amalgam removed from the cavity before hardening. A sharp-pointed chisel was used to remove a triangular-shaped area, approximately 1 mm. wide and 1 mm. in depth, from the entire marginal area. This amalgam was analyzed for mercury. The remainder of the restoration was then removed, the large pieces crushed and aliquots taken for analysis. The method for mercury analysis was that of Crawford and Larson,[8] and Swartz and Phillips.[9]

One brand of alloy* was used throughout the study. The mercury-alloy ratio was standardized and trituration accomplished by means of a mechanical

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*Baker Co., Aristaloy.

amalgamator. The expressing of mercury was done with hand pressure only in a nylon squeeze cloth. All condensation was completed in less than 3½ minutes as it has been shown that the residual mercury is related to the time interval which elapses between trituration and condensation.[10]

Five different condensing instruments were employed:

(a) *Hand pressure*—using No. 1 and No. 2 Black amalgam condensers,
(b) *Vibratory*—using a handpiece activated condenser (Kerr),
(c) *Impact*—using a handpiece activated condenser (Mid-West),
(d) *Pneumatic*—using a compressor activated condenser (Clev-Dent),
(e) *Pneumatic* with a rubber tip—essentially the same as above except that the overpack and marginal areas were further condensed using a special rubber tip (Clev-Dent) on the condenser.

Two series were run with each condenser. One series, designated as all dry, used amalgam in which the entire mass had been wrung dry before the introduction of the increments into the cavity. Care was taken, however, to never use increments from which some mercury could not be expressed during condensation.[4, 5] The other series employed the "increasing dryness" technic in which the mass was divided into increments and each increment wrung progressively dryer. Again care was taken to avoid increments which were either too wet or dry.[7]

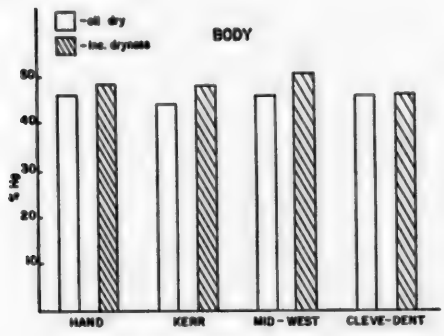

Fig. 1.

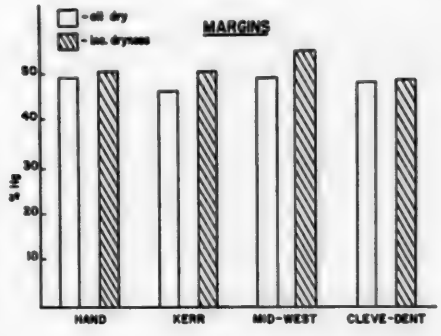

Fig. 2.

RESULTS

The mercury content in the body of the restoration is evident from Fig. 1. There is little difference in the results obtained with the various instruments but the mercury content is generally slightly higher when the increasing dryness technic was employed as compared to dry increments throughout. This finding is in contrast to the previous study[7] where the entire specimen was analyzed. However, the differences here are minimal and at these levels would have little effect on the physical properties.[9] The data in Fig. 2 indicate that the mercury content at the margins, as influenced by the various technics, was comparable to the results found in the body of the restoration but the mercury was consistently higher in this area. This finding corroborates previous findings.[9]

When the rubber tip is used to complete the condensation at the occlusal surface (Fig. 3), the mercury in both the body of the restoration and at the margins is very slightly reduced. However, the difference could not be considered significant.

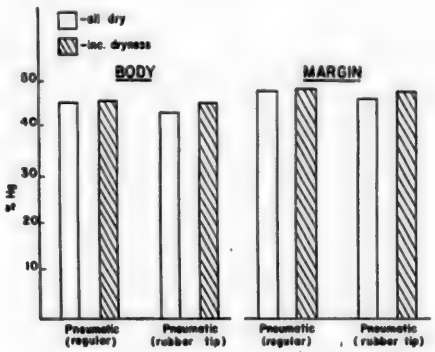

Fig. 3.

Table I is a summary showing the average per cent difference in the mercury content of the body and at the margins with the various methods of condensation. The increase in mercury at the margins was essentially the same for all technics except for the impact type of condenser when used with the increasing dryness technic. The particular method produced somewhat greater differences and calculations of the standard deviation showed the individual specimens to be more variable.

TABLE I

MEAN DIFFERENCE IN MERCURY CONTENT AT MARGINS AND BODY OF RESTORATION WITH VARIOUS CONDENSATION INSTRUMENTS AND WITH TWO TECHNICS—ONE USING DRY INCREMENTS THROUGHOUT AND THE OTHER EMPLOYING THE "INCREASING DRYNESS" PROCEDURE

CONDENSER	METHOD	AVERAGE DIFFERENCE BETWEEN BODY AND MARGIN IN PER CENT
Hand	All dry	2.6
	Inc. dry	2.3
Vibratory (Kerr)	All dry	2.4
	Inc. dry	2.7
Impact (Mid-West)	All dry	2.9
	Inc. dry	4.0
Pneumatic (Clev-Dent)	All dry	2.4
	Inc. dry	2.2
Pneumatic (rubber tip)	All dry	2.7
	Inc. dry	2.5

The results obtained in this study substantiate previous opinions that various technics, carefully controlled, will produce essentially the same final mercury content in the restoration and that regardless of the technic employed, the mercury concentration is always somewhat higher at the marginal areas.

SUMMARY

A study was made to determine the final mercury content at the margins and in the body of amalgam restorations when condensed with various instruments. Two different procedures, in terms of the mercury present in the individual increments, were used.

Regardless of the method of condensation or the dryness of the increments used to build the restoration, the marginal area of the amalgam restoration contained a higher per cent of mercury than did the bulk of the restoration. The residual mercury was slightly higher when the increasing dryness technic was employed.

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FURTHER STUDIES ON THE CONTOUR OF CLASS II RESTORATIONS WITH VARIOUS MATRIX TECHNICS

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IN a previous report[1] on the proximal contour of class II proximo-occlusal amalgam restorations it was shown that, using hand condensation, regardless of the matrix procedure employed, failure to wedge the band resulted in cervical overhang. In addition, it was found that with square or ovoid-type teeth optimum results in reproducing the original proximal contour were obtained by contouring as well as wedging the band.

Since mechanical devices have often been recommended and are widely used for condensing the amalgam restoration, it seemed advisable to study the effect of these instruments upon the contour and overhang with various matrix procedures. Certain other variables, such as the effect of flossing the proximal surface, were also investigated.

MECHANICAL CONDENSERS

Since the previous work had shown that reproducible and acceptable results were easily obtained on the tapering-type tooth without the necessity of contouring the band, it was decided to use this type. If the mechanical condensers did alter the contour, the change would be easily seen. The method of measuring the surface contour was the same as that previously used.[1] It consisted of silhouetting the proximal surface of the restoration and comparing this contour with the original. Overhang is measured both by these silhouettes and by direct photographs taken of the proximal surface.

The following matrix retainers and technics were selected as being representative and were used with all condensers: (1) Ivory No. 8, (2) Toffelmire, and (3) Miller's technic, which employs a wedged 0.002 inch matrix band supported with compound.[2] With the Ivory and Toffelmire retainers, restorations were prepared both with the band wedged and unwedged. Three different mechanical devices were used for condensing restorations with all of the matrix technics. They were the Kerr, Mid-West, and the Clev-Dent. The Kerr condenser is a vibratory type while the other two are impact condensers. With the Clev-Dent instrument, restorations were prepared using 2 different

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adjustments of the condenser—medium and heavy. A minimum of 4 restorations were prepared for each test condition and the results presented are the mean.

Results for the Kerr and Mid-West condensers are shown in Figs. 1 to 3. For comparison, the contours and overhangs produced by hand condensation are also included. The data obtained with the Clev-Dent are plotted in Figs. 4 and 5. (These curves do not represent the actual contour of the proximal surface; rather they depict the deviation, plus or minus, from the original at specific points on the occlusocervical axis. The gingival margin on this tooth is at position 10; thus values below this point represent the mesiodistal overhang. The overhangs, plotted at the right of each chart, depict the excess from the occlusogingival direction. The reader is referred to the first publication for more detailed description of the method of analysis.[1])

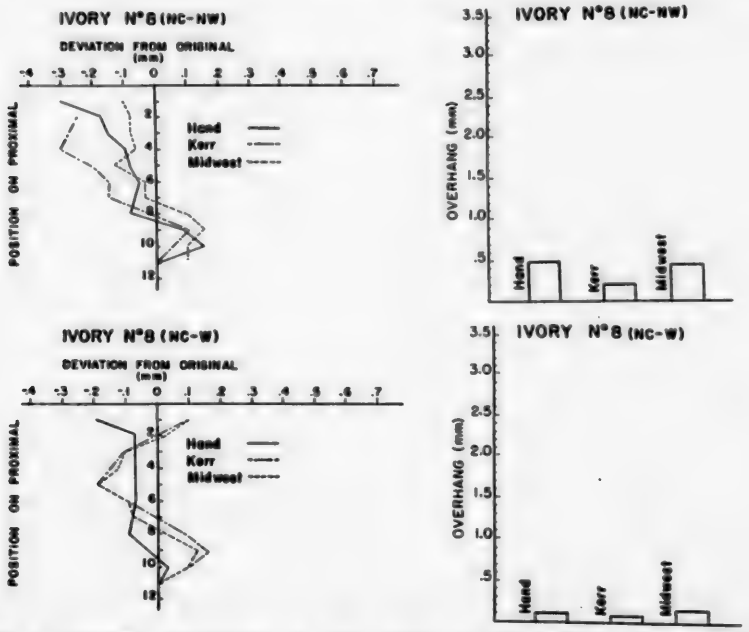

Fig. 1.—Contour reproduction of tooth, shown at left, with 3 condensation technics and Ivory No. 8 retainer. *NC-NW* = not contoured, not wedged; *NC-W* = not contoured, wedged. Curve depicts deviations, plus or minus, from original normal contour. Occlusogingival overhang is plotted at right.

The contours obtained by the mechanical condensers, with the exception of the Clev-Dent when heavy pressure was used, were comparable to hand condensation. The heavy impact with the Clev-Dent showed a tendency to displace the band when it was unwedged, particularly with the Ivory No. 8 retainer.

Comparison of the overhangs indicated that the Kerr condenser produced slightly superior results while the Clev-Dent with heavy pressure caused the greatest overhang. The advantage of wedging the band, irrespective of the condenser employed, is obvious.

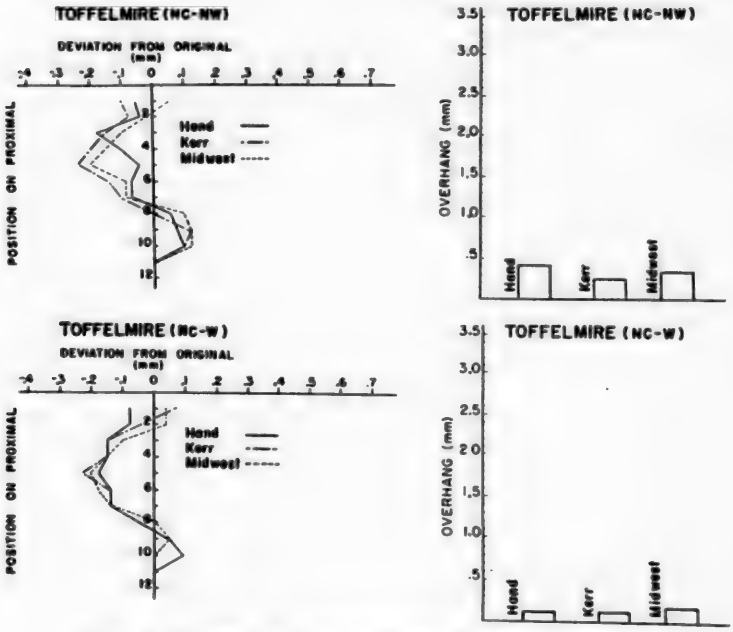

Fig. 2.—Contour and overhang with Toffelmire retainer.

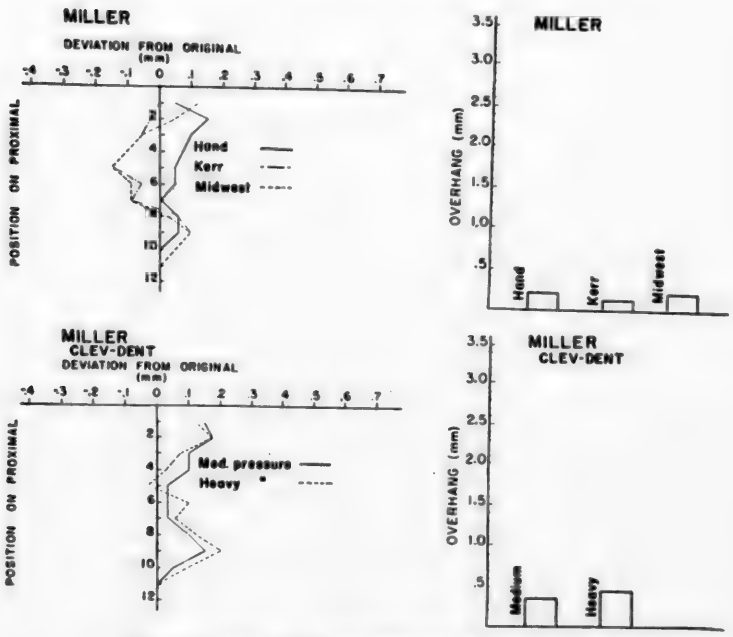

Fig. 3.—Contour and overhang with Miller technic.

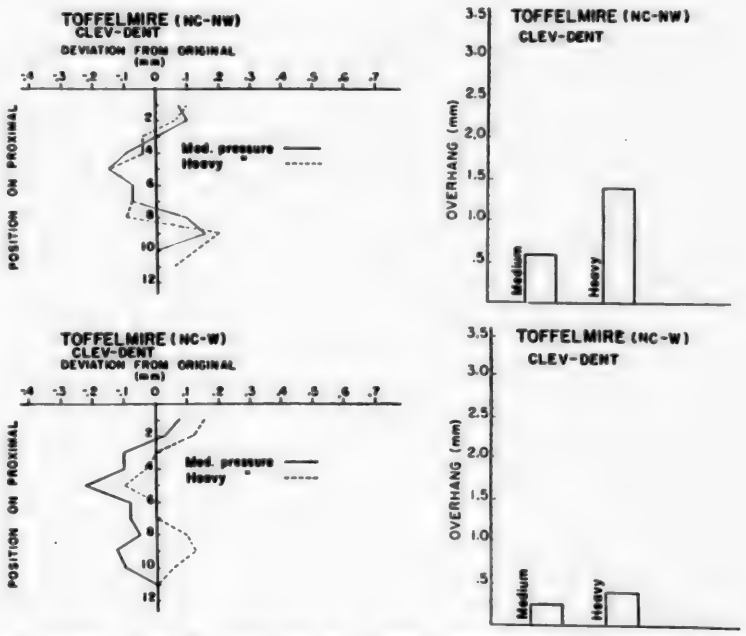

Fig. 4.—Results with 2 adjustments of impact condenser and Toffelmire retainer.

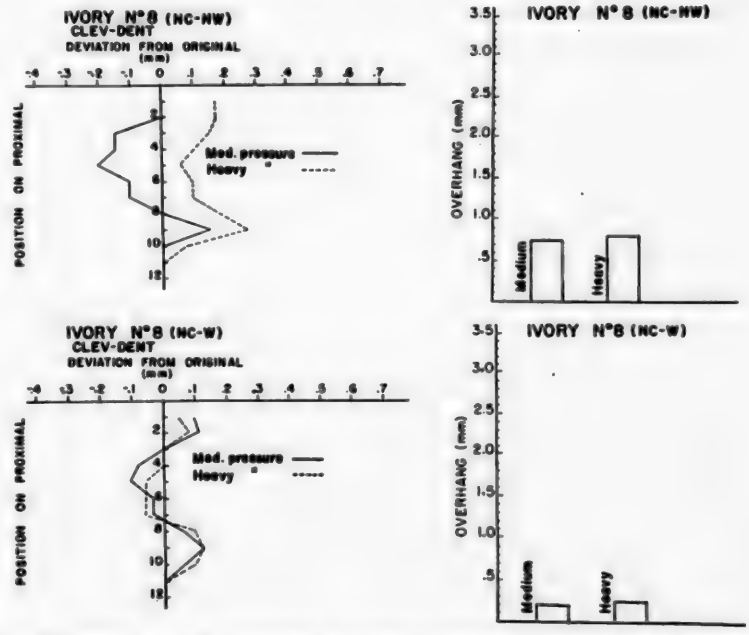

Fig. 5.—Results with 2 adjustments of impact condenser and Ivory No. 8 retainer.

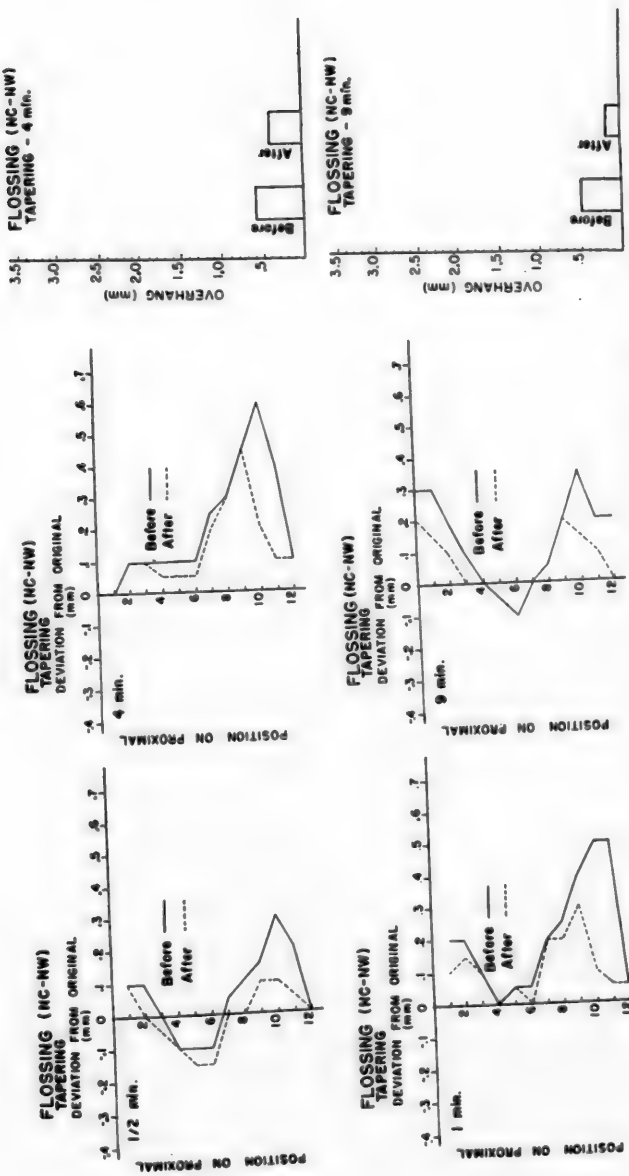

Fig. 6.—Effect of flossing proximal surface at various time intervals, tapering tooth. Contour is altered at gingival (position 10). Overhang, at right, is reduced slightly by use of floss.

In summarizing this phase of the study, it may be seen that generally, for a given matrix technic, contour of the restoration and the amount of overhang are comparable with either hand or mechanical condensation. Except for the Clev-Dent, heavy pressure, the mechanical condensers did not cause inferior contour. However, wedging of the band was even more important with these devices than when hand condensation was used. The vibratory condenser produced slightly smaller gingival overhangs than either of the other impact condensers or hand condensation but the difference was slight and probably only of academic interest.

EFFECT OF FLOSSING

This investigation has clearly shown that regardless of matrix technic or condensing instrument, wedging of the band is essential if the gingival overhang is to be minimized. Although the value of wedging cannot be overemphasized, the complications associated with application of the wedge do involve problems which may be difficult to control.[3, 4] Thus, many dentists prefer to omit the wedge and then attempt to remove the gingival flash and produce a more desirable proximal embrasure by passing floss through the proximal of the newly formed restoration. It was decided that the efficiency of this procedure should be studied.

Since the greatest flash had been produced by the use of an unwedged and uncontoured band, this particular technic was used as the method for preparing restorations on which the effects of flossing would be studied. Both tapering and ovoid teeth were restored with amalgam by hand condensation using uncontoured and unwedged bands and an Ivory No. 8 retainer. Immediately after condensation was completed, the proximal contour was silhouetted. Floss was then passed through the proximal contact and pulled through the gingival area. After the floss had been used, the contour was again photographed and compared with the original contour to determine how much, if any, of the excess was removed. Since the setting time of the alloy would influence the amount of overhang removed, tests were made at various periods of time (ranging from one-half minute to 10 minutes) from the time the matrix was removed until floss was used.

In Fig. 6 can be seen the results on the tapering tooth. (Because of the time factor in taking the silhouettes, the occlusogingival overhang could be measured only after 4 minutes.) It is apparent that the use of floss at any time interval altered the proximal contour only slightly in the area of the greatest convexity, position 3, of the tooth but it did aid considerably at the gingival, position 10. Likewise there was some reduction in the cervical overhang as shown by the graphs at the right of Fig. 6.

With the ovoid tooth, Fig. 7, use of floss at one-half minute or 1 minute did improve the proximal contour somewhat but when employed at the 4- and 9-minute intervals, no significant change was noted in either contour or overhang.

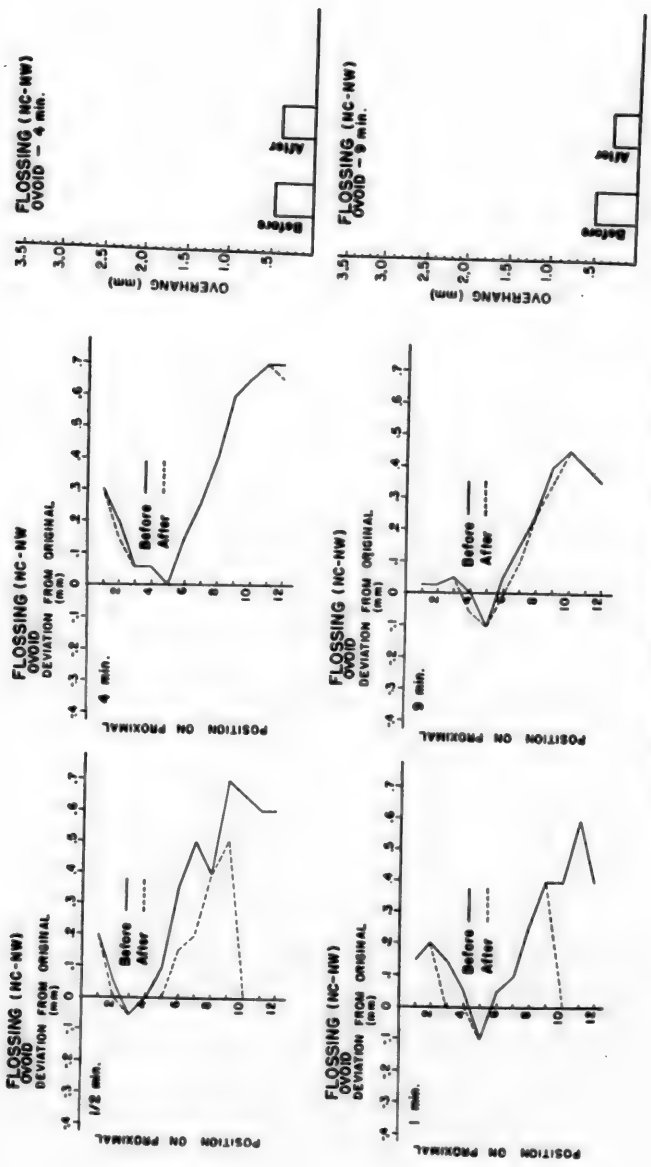

Fig. 7.—Effect of flossing using ovoid tooth.

It is thus obvious that if floss is to be effective in improving contour and removing the gingival overhang produced by an unwedged band, then, particularly with the ovoid tooth, it must be employed as soon as possible after removing the band. As the amalgam hardens it becomes increasingly difficult to remove the excess material. Even with optimum results, when the best flossing technic is compared to the contour produced by a contoured and wedged band (Fig. 8) there is more deviation from the original contour and the overhang is greater.

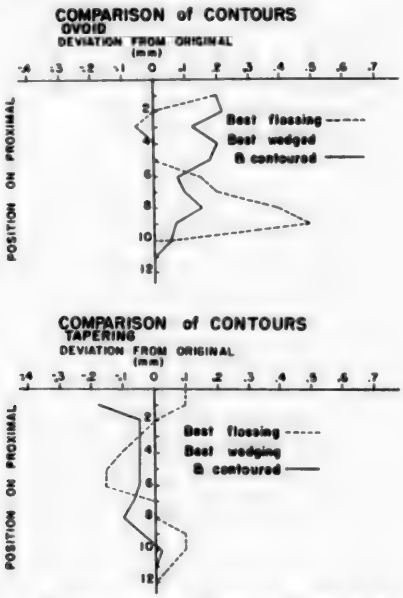

Fig. 8.—Contour reproduction with wedged and contoured band as compared to unwedged band followed by flossing.

TOTAL MERCURY WITH VARIOUS MATRIX TECHNICS

The MO preparation on the square-type molar was used. Five different matrix technics employed in previous work were utilized: (1) Toffelmire—wedged, (2) Toffelmire—not wedged, (3) Toffelmire—wedged and contoured, (4) Markley's technic, and (5) Miller's technic. All technics were employed in conjunction with 3 types of condensing instruments: (1) hand, (2) Mid-West, and (3) Kerr.

One alloy* was employed throughout. To insure uniformity of the mixes, the mercury-alloy ratio was standardized and trituration was accomplished by means of a mechanical amalgamator.[5] The "increasing dryness" technic was employed for condensation. It has been shown that this method produces satisfactory and uniform physical properties.[6] The buccal and lingual proximal margins were trimmed and marks were placed on the restoration parallel to the cervical and proximal margins with a pair of dividers. The width of

*Baker Co., Aristaloy.

this marked area was 1 mm. These triangular-shaped marginal areas were then carefully removed, by means of a chisel and were analyzed. A mass was also cut from the middle of the restoration and mercury content determined. In this way, relatively uniform samples from the representative areas of the restorations were secured for analysis. The method of mercury analysis employed was that of Crawford and Larson.[7] Ten restorations were placed with each procedure and the mean data are presented.

The mercury contents in the different areas of the amalgam restorations, when the various matrix technics and condensing instruments were utilized, appear in Tables I, II, and III. The standard deviation was calculated for each group in order to ascertain the amount of variation in individual specimens.

TABLE I

MID-WEST CONDENSER

TECHNIC	WEDGED	NOT WEDGED	WEDGED AND CONTOURED	MARKLEY'S	MILLER'S
Ave. % Hg mass	48.23	48.33	48.76	47.51	48.20
Ave. % Hg cervical	50.57	51.77	50.38	49.74	50.18
Ave. % Hg proximal	50.64	51.19	50.80	50.04	50.18

TABLE II

KERR CONDENSER

TECHNIC	WEDGED	NOT WEDGED	WEDGED AND CONTOURED	MARKLEY'S	MILLER'S
Ave. % Hg mass	48.72	49.31	48.21	49.22	48.40
Ave. % Hg cervical	51.37	50.67	49.30	51.12	50.35
Ave. % Hg proximal	51.35	50.82	50.45	51.87	50.89

TABLE III

HAND CONDENSER

TECHNIC	WEDGED	NOT WEDGED	WEDGED AND CONTOURED	MARKLEY'S	MILLER'S
Ave. % Hg mass	48.79	48.92	48.82	48.52	48.82
Ave. % Hg cervical	51.24	51.99	50.21	49.97	50.21
Ave. % Hg proximal	52.38	52.56	50.29	50.93	50.29

These data indicate no matrix technic, in conjunction with a specific condensing instrument, produced restorations that were superior in terms of final mercury.

It is interesting to note that the concentrations of mercury found in both the proximal and cervical marginal areas were always greater than that of the bulk of the restoration. These differences, ranging from 1.36 per cent to 5 per cent in most cases, were well above the calculated standard deviation. The data show that there is no significant difference in the final mercury content, particularly at the cervical, between the wedged and unwedged band. However, some interesting results were obtained when the cervical flash itself was analyzed. The mercury content of the mass of the restoration and that of the cervical overhang trimmed from restorations where the matrix

band was not wedged is shown in Table IV. The overhang was found to be rich in mercury, and averaged 7.21 per cent higher than that in the bulk of the restoration. Thus, it is apparent that in the unwedged band, the cervical overhang is extremely high in mercury, regardless of the condenser used. This finding is another sound argument for the wedged band since the overhanging amalgam created with the unwedged band would have all the deleterious properties associated with excess mercury.

TABLE IV

Hg CONTENT OF MASS AND OVERHANG

CONDENSERS	MID-WEST	KERR	HAND
Overhang mass	55.67	56.10	56.41
	48.33	49.31	48.92
Ave. diff.	7.34	6.79	7.49

CONCLUSIONS

One vibratory-type condenser and 2 impact types were studied to determine the effect of mechanical condensation technics on the contour of class II amalgam restorations using various matrix band procedures. Results were comparable to those of hand condensation and emphasize the necessity of wedging the band to avoid gingival overhang. Use of heavy pressure with one type of condenser increased the overhang.

The influence of flossing upon contour and overhang was investigated. Use of floss immediately after condensation does improve the contour and reduces the overhang produced by an unwedged band but is not as satisfactory as the wedged band. Use of floss approximately 4 minutes following condensation is of little or no value.

Analyses for mercury in restorations placed with various technics indicate that the mercury in both the proximal and gingival areas was always higher than in the bulk of the restoration. The differences ranged as high as 5.0 per cent. No particular technic or condenser was superior. Although the unwedged band did not produce an increase in mercury at the gingival, when the excess flash was analyzed an average increase of 7.2 per cent was found.

The authors wish to acknowledge the capable assistance of Dr. Paul Cleeter throughout this investigation.

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STUDIES ON SYNERESIS AND IMBIBITION IN REVERSIBLE HYDROCOLLOID

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INTRODUCTION

REVERSIBLE hydrocolloid is unexcelled as an impression material in the construction of inlays, crowns, and partial dentures.[1-4] Its use, particularly for indirect work, is ever increasing. Although hydrocolloid is conceded to be one of the most accurate of the impression materials, distortion and subsequent failures do occur. Many factors influence the accuracy that is possible with these materials and much research has been devoted to the influence of manipulation upon the physical properties.[5]

Possibly the most common cause for error associated with the clinical and laboratory use of hydrocolloids is the distortion of the material following removal of the impression from the mouth and the pouring of the working model. Hydrocolloid gel is approximately 80 per cent water and is very susceptible to syneresis (loss of water) and imbibition (gain in water) when exposed to different environments.[6] Unfortunately loss or gain of water in the material is accompanied by dimensional changes of clinical significance. Likewise it is believed, but has never definitely been proved, that a stress pattern is created during manipulation of the material and that storage of the impression for any length of time permits release of the internal stress and results in distortion.

Thus, dimensional instability of the hydrocolloid impression is a real problem and a limiting factor in the use of this type of material. Although most of the inherent properties of hydrocolloid are desirable ones, failure to pour the die immediately after the impression is secured leads to distortion and failure. Often it is impossible or inconvenient to pour the impression at that time, but as yet no means of preserving the dimensional stability of the impression has been established. This factor, probably more than any single one, contributes most to the success or failure of the material.

The effects of imbibition and syneresis on dimensional stability have been studied by numerous investigators. These investigations have included the dimensional change of the unconfined materials when floated on mercury[7] and in a trough,[8-10] changes when confined in a typical impression tray,[11] and measurement of stone models poured from hydrocolloid impressions.[12, 15] Other

Portions of this paper were presented at the 34th annual meeting of the International Association for Dental Research, March 22, 1956, St. Louis, Mo.

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studies have made use of the "fit" of castings fabricated on dies.[11, 16] Most of this research has included the influence of storage and of storage environment and all have emphasized the distortion which results from fluctuations in water within the impression.

However, none of these investigations has actually measured the water content in the impression and the water fluctuations which may occur during storage or manipulation. That was the purpose of this study.

PROCEDURE

Several standard chemical methods of moisture determination were considered and tested. The procedure found most applicable and accurate for measuring water in the gross amount that is present in hydrocolloid materials was a direct method involving weight loss of the specimen. The hydrocolloid was dried in a constant temperature oven at 105°C ± 5° and the weight loss determined. Preliminary tests were run to determine a satisfactory drying time (Table I). It was found that extension of the drying period beyond 48 hours did not result in further significant water loss. Thus, this time was selected as the drying period.

TABLE I

WATER CONTENT AFTER VARYING DRYING PERIODS

SAMPLE NUMBER	LENGTH OF DRYING TIME			
	48 HRS.	72 HRS.	96 HRS.	120 HRS.
1	84.82	84.84	84.86	84.89
2	84.81	84.80	84.85	84.89
3	84.52	84.57	84.63	—
4	84.50	84.51	84.52	—

Four popular commercial brands of hydrocolloid (Table II) were employed in the study. The products were handled according to the manufacturer's directions. A mixing gun was used as a matter of convenience and because it was felt this might result in a more homogeneous material.

TABLE II

MATERIALS USED IN THIS INVESTIGATION

BRAND	BATCH NUMBER	DATE PURCHASED
Deelastic	MM312-594	1/16/56
Van Rossem	5503192	1/16/56
Dentocoll	32-55-9B	1/16/56
Surgident	5553094	1/16/56

The specimens were prepared by filling No. 000 Coor's crucibles (6 ml. capacity) with hydrocolloid. A glass cover plate was immediately placed on the crucible and the excess material allowed to flow over the sides. After gelation, this excess was removed from the outside of the crucible, the cover glass taken off, and the crucible containing the sample immediately placed in the selected environment. The storage media employed in this study were air, distilled water, 100 per cent humidity, and 2 per cent potassium sulphate. Room temperature was 78°F. ± 5°. The area of the top surface of the mold which was exposed to the various media was approximately 0.69 sq. in.

One specimen taken from the center portion of the hydrocolloid in the mixing gun was immediately cut into small pieces, placed in a tared weighing bottle and the weight determined. An analytical balance was employed for all weighing and weights were determined to the nearest 0.1 mg. After the specimen had remained 48 hours in the oven, it was again weighed and the per cent moisture content calculated. This sample served as the control, since analysis of 8 tubes of hydrocolloid, 6 crucibles filled from each tube, exhibited marked uniformity. It was thus felt that one sample taken from each gun provided sufficient control.

For all environments except air, the surface of the specimen was lightly blotted with bibulous paper to remove excess moisture at the end of the designated storage period. Several methods of drying were tested. The data in Table III show that the method of drying the surface was not critical. After the samples had been blotted they were cut into small pieces and placed in tared weighing bottles and the water content determined.

All tests were made in triplicate and the data presented are the mean.

Water Changes in Hydrocolloid as Related to Storage Media.—

The effects of storage environment on the water content of specimens prepared from the 4 brands of hydrocolloid were studied. The average changes induced by storage in air, water, 100 per cent humidity, and 2 per cent potassium sulphate as related to the storage time appear in Figs. 1 to 4.

All specimens exhibited marked loss in water when allowed to remain in air, the magnitude of the change being dependent on the length of storage. This method of storage, of course, resulted in more rapid and greater changes in moisture than that of any other media. After only 1 hour in air, all but 1 hydrocolloid exhibited changes twice as great as those which occurred with other storage methods. The differences became more pronounced as the storage time was extended.

Analysis of those specimens stored in distilled water revealed a definite imbibition of water by the hydrocolloid, although neither the rate nor the magnitude of the change was so great as that produced by storage in air. Generally imbibition proceeded rather rapidly when the material was first placed in water, but the rate declined with time.

TABLE III

EFFECT OF VARIOUS METHODS FOR DRYING SURFACE BEFORE WATER ANALYSIS

METHOD OF DRYING SURFACE OF SPECIMEN	H_2O CONTENT (PER CENT)
Light blotting	87.59
Heavy blotting	87.47
Extreme heavy blotting	87.46
Weak air pressure	87.59
Strong air pressure	87.50

Samples stored in H_2O.

The data indicate that 100 per cent relative humidity is superior to the other storage media tested. The fluctuations were exceedingly small and for

the most part were in the magnitude of 0.1 to 0.2 per cent. The maximum change observed in any single specimen was 0.4 per cent after a 24-hour storage period.

The behavior of individual brands of materials in 2 per cent potassium sulphate was noted to be more erratic than in the other 3 environments. One

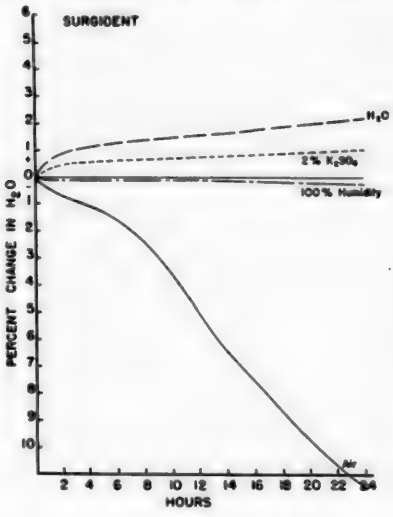

Fig. 1.

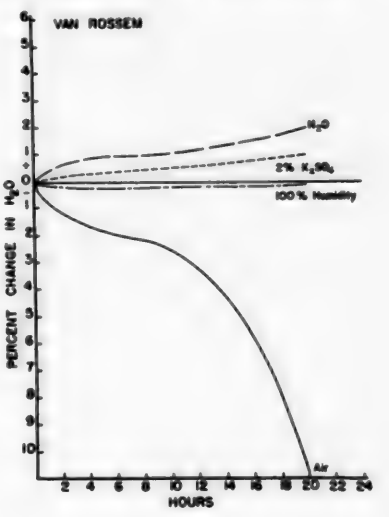

Fig. 2.

Fig. 1.—Water change in Surgident in various storage media.
Fig. 2.—Water change in Van Rossem hydrocolloid.

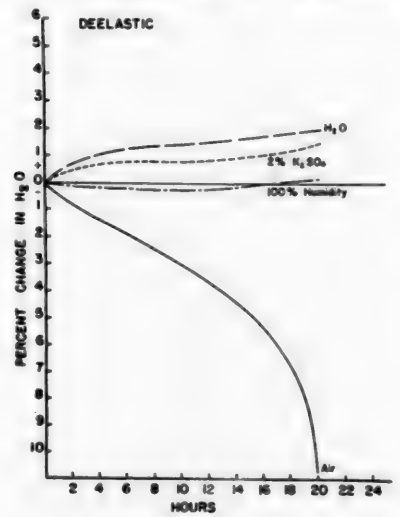

Fig. 3.

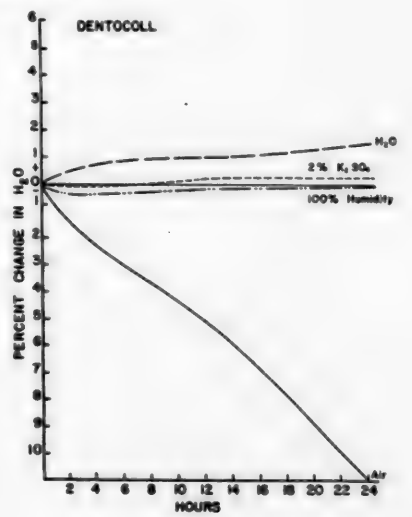

Fig. 4.

Fig. 3.—Water change in Deelastic hydrocolloid.
Fig. 4.—Water change in Dentocoll hydrocolloid.

brand, Dentocoll, appeared to be exceedingly stable in this medium, while the others exhibited varying degrees of stability. However, all show an increase in water after 20 or 24 hours of storage.

Alterations in Water Concentrations in Various Areas of the Specimen.— The changes which had been observed in the total water content of the entire specimen were relatively small, when stored in 100 per cent humidity or when stored for only 30 minutes in 2 per cent potassium sulphate. However, previous work on practical dies has shown that significant dimensional changes, in short periods of time, are produced under these same conditions.[12] It would

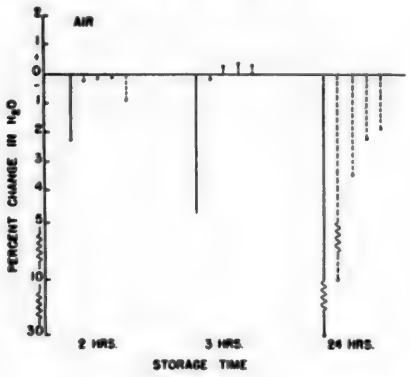

Fig. 5.

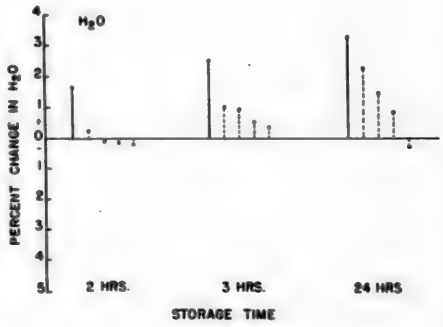

Fig. 6.

Fig. 5.—Water change, at different time intervals, in various areas of the hydrocolloid when stored in air. Solid line at left is outer section with each succeeding line representing progressively deeper areas.

Fig. 6.—Water change in various areas when stored in distilled water.

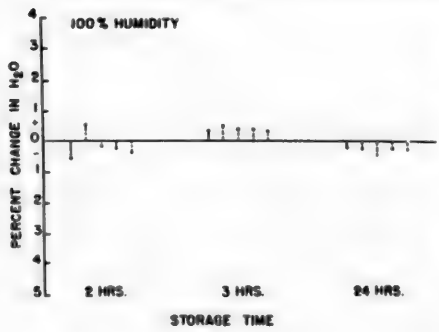

Fig. 7.

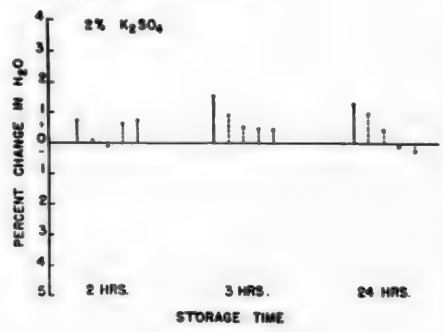

Fig. 8.

Fig. 7.—Water change in various areas when stored in 100 per cent humidity.
Fig. 8.—Water change in various areas when stored in 2 per cent K_2SO_4.

seem logical to suspect that the exposed surface of the material would be that most vulnerable to changes in water content. Thus, it is possible that water fluctuations might be much greater on the surface and analysis of the entire specimen would not indicate the effect on this critical area. For this reason it was felt that information should be obtained relative to water change at different levels in the mold. A method was devised for sectioning the molds of hydrocolloid with subsequent analysis of each portion. Immediately after

the specimens were taken from their respective storage environments they were carefully removed from the crucible and placed in a special slotted mold. By use of a razor blade, each mold was then cut into 5 sections. The thickness of each section was approximately 1/8 inch. Each section was analyzed for water content.

Immediately following gelation, a control specimen was taken from the center of the mixing gun, sectioned, and analyzed. Thus, information was obtained concerning the normal variation in water content in various areas of the mold and the water loss from the sectioning procedure.

The alterations in water concentrations at the different levels in the hydrocolloid as influenced by storage media may be seen in Figs. 5 to 8. The data obtained with storage in air, water, and 2 per cent potassium sulphate clearly indicate that the greatest fluctuations do occur in those areas in contact with the storage environment. Only after prolonged storage did the deeper layers show significant changes in water concentration. No definite difference could be detected at the surface of specimens stored in 100 per cent humidity. It is probable that any differences which might have occurred were so slight as to be obscured by the variations in moisture content of the individual sections.

The changes noted here by all methods of storage compare with those obtained in the previous series of tests using the entire specimen. Again 100 per cent humidity proved to be the environment which maintained water stability most efficiently, followed by 2 per cent potassium sulphate. Gross changes were observed in the moisture content at the surfaces of specimens placed in either water or air.

DISCUSSION

This paper must be considered as a progress report with many factors remaining to be investigated. However, a workable technic has been established for actually measuring the water content of these materials and determining the magnitudes of change as influenced by certain manipulative variables.

It must be recognized that there are slight variations in water in different areas of the hydrocolloid even when the mixing gun is employed. Preliminary data indicate that these variations may account for some slight occasional inconsistencies noted in individual specimens. Likewise, variations will undoubtedly occur in different batches of material. The trends are definite, however, and most certainly the water content of the materials is altered to some degree by storage in any media and the greatest change occurs in the surface areas exposed to the storage environment.

It is interesting to note that the behavior of hydrocolloid in each medium compares rather well with earlier observations which have been concerned with dimensional stability. The storage media in which the smallest dimensional changes occurred were those which produced the least alterations in the water content. The reverse was of course true. Although it has been shown that storage in 100 per cent humidity produces less distortion of the impression than storage by any other method, it has also been demonstrated[5] that allowing an impression to remain in this medium for even 30 minutes before pouring the die may result in distortion of clinical significance. The extremely slight, in fact almost negligible, moisture changes manifested by materials stored in this

medium would suggest that further work be done to determine the relative importance of moisture content per se in governing the stability of the hydrocolloid impression at these early times. To permit a complete evaluation, future studies must determine whether these very slight alterations occurring in either 100 per cent humidity or 2 per cent potassium sulphate are primarily responsible for distortion or whether, as suggested by Hampson[7] and others, this distortion is due to the release of strain or is a combination of both factors.

These things along with many others must be determined before the practicability or the value of attempting to compound isotonic storage solutions can be determined.

SUMMARY

1. A method for quantitatively determining the changes in water content of reversible hydrocolloid materials in various storage media has been described.

2. Changes were induced by storage in any medium with the magnitudes being dependent on the nature of the environment and the storage time. Only slight changes in moisture were observed when materials were stored in 100 per cent humidity. Generally the materials were not as stable in 2 per cent potassium sulphate as in humidity; however, the stability varied with the different brands of hydrocolloid employed. Specimens stored in distilled water exhibited definite increases in moisture content, while the water content of hydrocolloid allowed to remain in air was markedly reduced.

3. As would be expected the greatest portion of these changes occurred at the surface of the specimens with the deeper layers being affected to a lesser extent and only after prolonged storage.

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AN EVALUATION OF LUBRICANTS FOR DENTAL HANDPIECES AND CONTRA-ANGLES

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SINCE the advent of increased speeds of operation for the shaping of cavities, the problems associated with the care and maintenance of dental handpieces have become more significant.[14, 21] One of the most important is proper lubrication. The suggested materials and methods for the lubrication of handpieces and contra-angles are numerous and apparently many have been chosen rather empirically.

Proper care of the dental handpiece and contra-angle involves (1) cleaning, (2) sterilization, (3) lubrication, and (4) adjustment. It seems, therefore, that an evaluation of the lubricating properties of a representative group of lubricants would be the first order of investigation into the general problem of care and maintenance of these instruments. The methods of cleaning and sterilization are, at least to some extent, dependent on the type of lubricant used, while the mechanical adjustment of the handpiece is common with, but independent of, these factors.

The literature contains numerous references, but little information of value on the lubrication of dental handpieces and contra-angles, and only an empirical evaluation of the methods described.[3-6, 8, 10, 11, 14, 17, 19, 20] Except for one of these articles,[14] the lubrication described is accomplished during some form of hot fluid sterilization. Various cleaners or solvents are mentioned,[6, 10, 19, 20] but no basis for their selection is reported.

In this study, investigations of lubricating fluids were made in 4 areas: (1) viscosity, (2) stability of the fluids under heat, (3) practical evaluation in the straight handpiece, and (4) practical evaluation in the contra-angle.

Viscosity.—The viscosity of a fluid is its resistance to change of form or its "internal friction,"[12] and is one of the most useful characteristics in determining a suitable lubricant.[16, 25] Lubricating oils of low viscosity were suggested by various refineries[1, 9, 23, 24] as being most suitable for use on the straight handpieces and contra-angles.

The viscosity of a lubricating fluid varies according to the temperature, and becomes thinner, with a greater tendency to flow at higher temperatures. The viscosity is generally defined by the oil industry in Saybolt Universal Seconds, which is the length of time for 60 ml. of oil to flow through a given tube orifice at a given temperature. Temperatures of 70° F., 100° F., and 130° F.

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were chosen at the beginning of this study to test the viscosity, since it was believed these probably represented the extreme temperatures of use; such as a cool office, and beginning use of the handpiece, and a heated handpiece becoming uncomfortable to both the operator and patient.

A Saybolt Standard Universal Viscosimeter was used to record the viscosities of the various fluids sold specifically for dental handpiece lubrication and those suggested by companies in the lubricative field. These lubricants are shown in Table I. The viscosities presented in Fig. 1 and 2 show an extreme variation in the mineral oil and synthetic lubricants suggested for the dental handpiece, with greater dispersion of viscosities of the various fluids at 70° F. than at 100° F. and 130° F. The mineral base lubricants marketed specifically for dental handpiece use range from 67 to 358 Saybolt Universal Seconds at 100° F. The only proprietary dental synthetic lubricant in Fig. 2 is a silicone, which has a viscosity of 387 Saybolt Universal Seconds at 100° F.

TABLE I

Mineral Oil Lubricants	
A. Prorex C, Socony-Vacuum Oil Co.	K.* Ritter Engine Oil, Ritter Co., Inc.
B. Gargoyle Velocite Oil No. 6, Socony-Vacuum Oil Co.	L. Cadet Oil B, Sinclair Refining Co.
C.* Midwest M. O. Lubricant, The Midwest Dental Manufacturing Co.	M. Prorex D, Socony-Vacuum Oil Co.
D. Tellus Oil No. 15, Shell Oil Co.	N. Tellus Oil No. 23, Shell Oil Co.
E. Corvus Oil, The Texas Co.	O. Hydra Oil, The Texas Co.
F.‡ AC_{10} (Dental), The Midwest Dental Manufacturing Co.	P.* S. S. White Lubricating Oil, S. S. White Dental Manufacturing Co.
G. Lily White Spindle Oil AX, Sinclair Refining Co.	Q.* Solubri Oil, The Cleveland Dental Manufacturing Co.
H.* Germilitol, Kerr Dental Manufacturing Co.	R. L-912, Shell Oil Co.
I.* Denslube, Densco, Inc.	S.* Young's Dripless Oil, Young Dental Manufacturing Co.
J. Finol, USP	T.† Kadex Sterilizer Oil, The William Getz Corp.
	U.† Pelton Sterilizing Oil, Pelton-Crane Co.
Synthetic Lubricants	
1. OS-45, Monsanto Chemical Co.	5. Ucon LB-140-X, Carbide and Carbon Chemicals Co.
2. WS-2824, Penola Oil Co.	6. P-35, Penola Oil Co.
3. P-16, Penola Oil Co.	7. Ucon LB-170-X, Carbide and Carbon Chemicals Co.
4. SD-17, National Advisory Committee for Aeronautics	8.† DC-550, Dow Corning Corp.

*Proprietary dental lubricating oils.
†Proprietary dental sterilizing fluids.
‡Proprietary dental emulsifying oil for sterilization.

The proprietary dental sterilizing fluids tested are much more viscous than those oils used purely for lubricating purposes. This seems proper, since the less viscous fluids tend to volatilize at the higher temperatures of sterilization. The hydrocarbon lubricants *L, M, N* (Fig. 1) suggested by 3 oil refineries[1, 23, 24] as being the most suitable for use as sterilizing fluids were found to have similar viscosities. Although the viscosities of 3 greaselike lubricants as listed in Table III were not recorded, it has been observed that greases will act as their basic oils at speeds of around 20,000 r.p.m. with the close tolerances used in the handpieces.[26, 29]

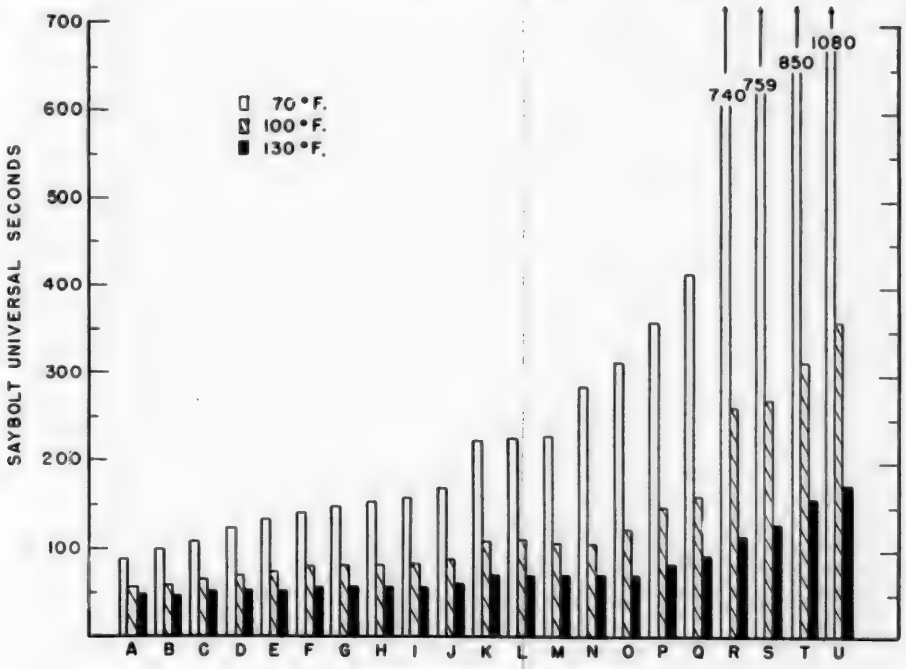

Fig. 1.—Viscosity—mineral oil lubricants.

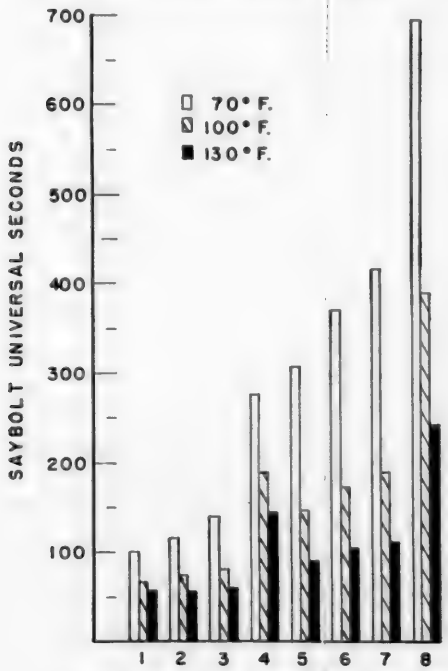

Fig. 2.—Viscosity—synthetic lubricants.

Stability of Fluids.—Many products are available to the dental profession which are advocated for use as a hot sterilizing fluid and lubricant. The oil refineries[1, 2, 9, 23, 24] contacted all expressed serious doubt that a straight mineral oil could be used for the purpose of sterilization at 300° F., because of the lack of thermal stability. A lubricant of suitable viscosity for the dental handpiece will generally be prone to give excessive volatilization at 300° F.[1, 2, 24] Three refineries,[1, 23, 24] however, submitted oils which they believed to be the most satisfactory for use for sterilization at 300° F.; the fourth[9] stated that a straight petroleum product would not be satisfactory. Some synthetic lubricants were suggested[23, 25] for testing as a sterilizing medium, since it was believed they might be more stable at the high temperatures of use.

To check the thermal stability of the various fluids, a Lindberg hot plate was used which has a uniform heating surface. Two-hundred-fifty milliliter Erlenmeyer flasks were each filled with 100 ml. of the fluid. Thermometers resting at the bottom of the flasks registered 300° F. for 50 hours. Odor and color were noted before and following the heating. The volume loss was also recorded. From Table II it is evident that of the mineral oil lubricants tested for thermal stability, those white oils of highest viscosity appear to be most stable in color and odor. Those oils which had slight yellow coloration before being heated had the greatest color change. The volume loss of the mineral base products ranged from 4.5 to 15.0 per cent and appears to be independent of viscosity, color, or odor. Color can be misleading as an indication of oil breakdown, however, since some high temperature antioxidants turn color quite easily on heating with no change in the base oil.[27]

Of the synthetic fluids, WS-2824, a diester, and SD-17, a silicone-diester blend, both produced a heavy sediment with the former also having an objectionable odor. OS-45 and tetra (2-ethylhexyl) silicate, both silicate esters, seemed to hydrolize in the presence of moisture. It was noted that when 2 per cent of water was placed in the fluid and the fluid held at 98° F. for an extended period of time, hydrolysis occurred resulting in the formation of a fine precipitate. Water added to the tetra (2-ethylhexyl) silicate at 250° F. resulted in the formation of a gel-like mass within a few hours. This precipitate was not soluble in benzene, carbon tetrachloride, or Stoddard Solvent. Such an occurrence in practice would exclude the use of this material, since greater abrasive wear might occur.

The UCON lubricant LB-170-X, a polyalkylene glycol, is essentially the same type of lubricant as LB-70-X, the difference being in the molecular weight of the polymer. The former has a viscosity of 170 Saybolt Universal Seconds at 100° F. while the latter is 70 Saybolt Universal Seconds at 100° F. The difference in volume loss should be noted, the less viscous material losing about five times as much as the other. The color change is also less with the LB-170-X. The odor of all the UCON products is satisfactory both before and following heating. During use in the sterilizer, a slight nonobjectionable odor of alcohol persists immediately over the surface of the fluid.

TABLE II

THERMAL STABILITY OF LUBRICANTS

PRODUCT	COLOR	ODOR AT ROOM TEMPERATURE	VOLUME LOSS (ML.)
Mineral Oil			
Pelton Sterilizing Oil	Clear white to light straw yellow	None to sweetish oil	9.0
Johnson Sterilizing Oil	Clear white to light straw yellow	None to slight oily	15.0
Kadex Sterilizer Oil	Light straw yellow to orange	Slight oily to slightly objectionable	7.0
Shell Tellus 23	Light straw to brownish black, sludge	Pungent oily to very objectionable	11.5
Sinclair Cadet B	Light straw to wine red	Oily to slightly objectionable	4.5
Socony-Vacuum Prorex D	Light straw to dark wine red	Oily to decidedly objectionable	5.5
Synthetic			
SD-17	Light greenish-yellow with slight dark sediment to deep reddish brown with much black sediment; reddish crystalization at neck of flask	Slight lacquer, unchanged	5.5
OS-45	Clear orange, unchanged	Characteristic pungent odor became objectionable during first 3-4 hours of heating, then disappeared; at room temperature, unchanged	7.0
UCON LB-170-X	Clear orange to clear red	Slight alcohol odor, disappears	3.5
WS-2824	Greenish orange with slight green sediment to deep reddish black with heavy sediment	Slight pungent to objectionable	4.0
UCON LB-70-X	Clear orange to dark reddish brown	Alcohol to none	17.0
UCON di (2-ethylhexyl) adipate	Very light clear orange to dark red	Slight alcohol to none	3.0
UCON 818-X	Clear orange to dark reddish brown	Slight alcohol to none	3.0
UCON HB-100-X	Clear orange to dark reddish brown	Very slight alcohol to none	5.5

Lindberg Hot Plate.
Temperature: 300° F.
Time: 50 hours.
Volume: 100 milliliters.

The DC-550 silicone has been in use for some time in the clinic at the School of Dentistry, University of Michigan, and has shown excellent thermal stability at 300° F.

A Pelton Hot Oil Sterilizer which operates at 250° F. was used to check the thermal stability of LB-170-X and Whip-Mix Sterilization Fluid No. 20 for 675 continuous hours. The LB-170-X had the same color change as noted when exposed to 300° F. for 50 hours. No objectionable odor was noted immediately over the surface of the fluid during heating or following. No objectionable odor was noted with the Whip-Mix fluid, but the color change was from clear white to deep wine red.

Although no viscosity measurements were run following heating of any of the fluids, it seemed apparent that an increase in viscosity did occur in all the fluids.

Practical Evaluation of Lubricants in the Straight Handpiece.—The lubricative ability tests were carried out directly on the handpiece under the conditions in which the lubricant would be used. It has been observed that bench tests give little or no reliable information concerning the lubricating ability of a product.[25] There is no laboratory research apparatus which reproduces, in all respects, the conditions under which the lubricant must perform.[15]

The properly adjusted straight handpiece was held in the middle area of the sheath by a clamp on a ringstand at a 20-degree angle from horizontal, with the nose end pointing downward. A mandrel was inserted through a ball bearing support from which a weighted pan was hung so that a load could be placed on the handpiece.

Chromel-alumel thermocouples were held in intimate contact with the sheath directly over the nose bearing and rear bearing by means of rubber elastics. The temperatures were recorded each minute for each bearing on a Leeds-Northrup Recorder during the 5-minute continuous run.

The handpiece was cleaned before each test run with Stoddard Solvent by using a small spiral brush, then blown dry with filtered compressed air, and the lubricant sparingly applied from the original container with a clean metal rod, while the handpiece was disassembled. The belt tension was properly adjusted for a particular handpiece, and all tests carried out for that handpiece, so far as possible, to eliminate the necessity of changing belt tension for each run. The belt tension was established by running the handpiece with the foot control in the highest position to establish the maximum spindle speed, as determined by a tachometer, and at the same time having the belt free from oscillations. With experience, this maximum speed can be approximated by ear, but should be checked with a tachometer to establish the speed of rotation. The belt tension thus established allowed a 7.5 to 8.0 cm. separation between the drive and return sides of the belt when a 200-gram weight was placed on the middle of the belt. The speed used was 15,400 ±600 r.p.m. A constant check was maintained on the belt tension.

It should be noted that the Chayes Model 3 handpiece was lubricated with Molykote and cleaned with the recommended cleaner according to the manufacturer's instructions. This was accomplished following the check tests made with other lubricants because of the nature of the Molykote lubricant and its tendency to adhere to the bearing surfaces even when a cleaner is employed.

The data in Table III represent the rise in temperature above the ambient temperature, measured directly over the bearings on the sheath at the end of the 5-minute run. Three new handpieces were used each with a 200-gram load.

The use of lubricants, not otherwise discarded for various reasons, shows that for most tests runs the nose bearing of the Chayes Model 3 dental handpiece ran cooler than did the nose bearings of the S. S. White No. 9 and Midwest Hi-Speed. However, the rear bearing of the Chayes handpiece ran consistently warmer than the rear bearing of the other 2 handpieces. The Chayes

rear bearing ran warmer than the nose bearing when the viscosity went above 150 Saybolt Universal Seconds at 100° F. This difference in temperatures developed is probably due to mechanical design and physical construction of the bearing surfaces.

For lubricants with a viscosity below 150 Saybolt Universal Seconds at 100° F., the temperature rise above the ambient temperature in the Chayes handpiece did not exceed 35° F. for either bearing. The highest temperature recorded was 61° F. for the rear bearing while using a silicone DC-550; the nose bearing temperature rise was 46° F.

The lowest temperature rise for the combination of both bearings was obtained with Prorex C, a Socony-Vacuum oil with a viscosity of 58 Saybolt Universal Seconds at 100° F. in the Chayes Model 3 handpiece.

TABLE III

PRACTICAL EVALUATION OF LUBRICANT IN STRAIGHT HANDPIECE

	VISCOSITY AT 100° F. (SUS)	°F. RISE ABOVE AMBIENT TEMPERATURE					
		S. S. WHITE NO. 9		MIDWEST HI-SPEED		CHAYES MODEL 3	
		REAR	NOSE	REAR	NOSE	REAR	NOSE
Mineral Oils							
S/V Prorex C	58	9	36	15	32	26	16
S/V Gargoyle Velocite	61	6	29	11	33	14	28
Midwest Lub.	67	8	44	22	47	26	33
Shell Tellus 15	71	15	39	16	41	25	32
Texaco Corvus	75	10	46	13	43	26	27
AC_{10}	78	12	42	21	33	29	30
Sinclair Lily White AX	82	7	33	21	31	30	30
Germilitol	84	15	36	18	38	28	30
Denslube	84	17	41	22	47	30	33
Finol, USP	91	20	45	8	35	28	28
Ritter Engine Oil	108	16	33	31	32	25	35
Texaco Hydra	125	10	35	13	38	24	32
S. S. White Lub. Oil	148	21	36	26	46	33	24
Solubri	160	24	44	30	45	38	34
Shell L-912	263	17	49	18	34	30	41
Young's Dripless	271	35	70	18	56	39	37
Kadex Ster. Oil	315	20	49	45	37	46	36
Pelton Ster. Oil	358	15	62	50	89	50	35
Pfingst Zenith Oil*		16	60	32	76	32	40
Johnson Ster. Oil*		50	49	52	100	33	47
Greaselike Lubricants (Mineral Base)							
S. S. White Handpiece Ease*		15	49	29	51	34	45
Densco Absorbed Oil*		13	47	37	59	35	45
Lubriplate*		19	73	40	55	34	52
Synthetic Lubricants							
UCON LB-70-X*		14	29	21	41	34	34
UCON 818-X*		23	34	24	36	32	25
UCON Adipate*		13	36	21	41	18	31
UCON 50-HB-100-X*		15	39	30	66	30	44
UCON LB-140-X	146	15	62	27	47	16	36
UCON LB-170-X	187	20	56	41	87	48	47
DC Silicone 550	387	57	92	50	92	61	46
DC Silicone XF-4140*		40	80	51	106	45	44
GE Silicone 81406*		18	113	42	87	51	34
Linde Air Silicone X-522*		43	77	54	93	28	51
Whip-Mix Ster. Fluid #20*		34	32	26	58	43	40
Molykote*				8†	88†	5	17

*Saybolt viscosity not determined on these products.
†Stopped run at end of 3 minutes.

The S. S. White No. 9 and Midwest Hi-Speed handpieces have similar rises in temperature over the bearings. In all test runs on the S. S. White No. 9 and Midwest Hi-Speed except one, the nose bearing heated more than the rear bearing, the ratio being as low as 31°/32° to a high of 8°/44°. The exception occurred with the Whip-Mix Sterilization Fluid No. 20 on the S. S. W. No. 9 where the ratio of temperature rise of rear and nose bearing was 34°/32°.

The S. S. White Handpiece Ease and Densco Absorbed Oil reacted similarly in each of the 3 handpieces. When using grease type lubricants, the temperature rise over the nose bearing varied from 45° F. to 73° F.; over the rear bearing from 13° F. to 40° F., being highest with the Midwest handpiece.

Although there is a general trend toward higher bearing temperatures as the viscosity of the lubricant increases, there are many fluctuations which occur.

The Densco Ball Bearing Handpiece was tested using Denslube on the nose bearing; the rear bearing is packed at the factory. At the end of 5 minutes with a 200-gram load there was a temperature rise of 8° F. and 10° F. over the rear and nose bearings, respectively.

Practical Evaluation of Lubricants in the Contra-Angles.—The lubricants used in the contra-angles were evaluated in essentially the same manner as was used for the straight handpieces. The straight handpiece was positioned in a plane 20 degrees to vertical so that the mandrel in the contra-angle was lying horizontally. The load thus placed on the contra-angle would be similar to that when a pulling force was applied to the handpiece during cavity preparation.

The thermocouples were placed at the head of the contra-angle and at the elbow. No water or air coolants were used with the Chayes WWCL. The results obtained are shown in Table IV.

TABLE IV

PRACTICAL EVALUATION OF LUBRICANT IN CONTRA-ANGLE

LUBRICANT	VISCOSITY AT 100° F. (SUS)	°F. RISE ABOVE AMBIENT TEMPERATURE					
		SSW-U		DENSCO UFS		CHAYES WWCL	
		ELBOW	HEAD	ELBOW	HEAD	ELBOW	HEAD
Prorex C	58	10	11	19	21	22	25
SSW Lub. Oil	148	17	21	25	30	25	21
Pelton Ster.	358	29	37	26	30	52	60
Densco Absorb.	—	26	29	23	26	40	51
50-HB-100-X	100*	16	21	26	32	40	54
Angle-Lube (grease with molybdenum disulfide)	—					45	47
81406 (silicone)	—	31	37			40	37

*Reported by Carbide and Carbon Chemicals Co.

Five representative lubricants from the low, middle, and high viscosity mineral base products, as well as a grease and synthetic fluid were tested with three contra-angles. A grease containing molybdenum disulfide, and a new silicone product were also checked in 2 of these contra-angles.

It is shown in Table IV that all 3 contra-angles ran progressively warmer as the viscosity of the mineral oil increased. The use of the greaselike lubricant resulted in slightly lower temperatures than with the most viscous oil, while the synthetic UCON lubricant reacted similar to the middle viscosity oil in the S. S. White and Densco contra-angles, and as the grease in the Chayes contra-angle. The head of the contra-angles operated at a slightly higher temperature than did the elbow in most of the test runs.

Cleaners.—

The gears and bearings of the contra-angles and straight handpieces may become contaminated with bits of some revolving instruments, cut tooth tissue, polishing material, and fluids during use in the mouth. Cleaning immediately after use, therefore, becomes essential, otherwise abrasive wear will tend to occur more rapidly.

Various cleaners have been advocated including carbon tetracholoride and various petroleum distillates. The carbon tetrachloride is generally not used industrially for the cleaning of precision machinery. This solvent, in the presence of moisture and light, liberates traces of hydrochloric acid which are very corrosive to metals.[7, 18] Statements are sometimes made that carbon tetrachloride leaves a residual film. This residual film upon examination has been proved to be either residual moisture (which the carbon tetrachloride does not remove) or particles of insoluble dust that adhere to the surface. Although carbon tetrachloride is a good solvent for most industrial lubricants, it does not touch some.[13]

Carbon tetrachloride has the advantage over any of the petroleum distillates of being noninflammable, although it has a fairly high toxicity. Of the petroleum products which appear suitable for use as a handpiece cleaner, Stoddard Solvent has a flash point (closed cup) of more than 20° F. higher than the others. Marsden[18] stated that Stoddard Solvent is almost identical with British Standard White Spirit and very similar to American "Mineral Spirit." Its flash point is 100° F. This solvent is generally not considered to present any industrial hazard due to its relatively low vapor pressure and it is not corrosive to metals. Sax[28] listed Stoddard Solvent as "safety solvent naphtha" which is a flammable liquid of moderate fire hazard, but not susceptible to spontaneous heating. Quadland[22] called Stoddard Solvent a high flash point petroleum solvent of low toxicity.

Stoddard Solvent was generally used during this investigation for the removal of the various lubricants. Its effectiveness, as judged empirically, was good. Solvents which meet the Standard Stoddard's specifications are inexpensive and readily available from petroleum distributors.

Since moisture may be a contaminator of the handpiece and contra-angle, and since neither the petroleum nor chlorinated solvents used are miscible with this moisture, difficulty might arise in obtaining suitable cleanliness of the instruments. It appears possible that some of this moisture, along with other contaminators, might be trapped in bearing crevices or between gear teeth.

This trapped material would become an undesirable residue. Thus, some method of dispelling this moisture and any suspended particulate matter would seem highly desirable.

DISCUSSION

Viscosity is obviously an important factor in the selection of a lubricant as is illustrated by the difference in temperature rise of the sheath directly over the bearings when using fluids of different viscosities. This temperature is probably of greatest practical interest to the dentist, since the temperature of the sheath will influence both his and the patient's reactions toward the handpiece and the life of the handpiece bearings.

It was noted that there was progressively closer agreement in viscosities when tested at 100° F. and 130° F. than at 70° F. This phenomenon can be graphically shown by projecting the measured viscosities on an A.S.T.M. Standard Viscosity—Temperature Chart. If the temperature rise of the sheath ranges from 6° to 113° F., the temperature rise at the interface of the bearings, or of the lubricant itself, probably is much higher, since a great amount of heat is dissipated throughout the surrounding area of the bearing. While the handpiece is in operation, the viscosity of the lubricants will be in closer agreement with each other than is indicated by the observations recorded in Figs. 1 and 2. At 70° F. the viscosities of the mineral base products range from 89 to 1080 Saybolt Universal Seconds; at 100° F. from 61 to 358 Saybolt Universal Seconds; at 130° F. from 52 to 170 Saybolt Universal Seconds; and when projected to 200° F., they range from about 35 to 60 Saybolt Universal Seconds. Therefore, there probably is some quality other than viscosity which influences the ability of a fluid to lubricate the dental handpiece.

Some of the lubricants obtained from the refineries contained antifriction additives while others were straight mineral oils. The presence of additives in the proprietary dental lubricating oils was not determined. It appears that the use of some nontoxic additive material to reduce friction and increase the load carrying ability of the lubricant might be employed to some advantage. More study of the nature of additives in lubricants for dental handpieces and contra-angles is indicated.

Certain of the more viscous white oils could be used as sterilizing or disinfecting fluids with some restrictions on the temperature and length of time used. Mineral base oils tend to become acid when they decompose.[26] The data show, however, that the use of such an oil would not provide sufficient lubrication at moderately high speeds of operation with light loads. Although the data presented showing temperature rises over the bearings were recorded at the end of a 5-minute run, it was noted that approximately 50 per cent of this rise occurred at the end of 1 minute and 75 per cent at the end of 2 minutes. Thus, it is probable that for relatively short interrupted runs, the temperature might readily become uncomfortable to the operator and patient.

Of the various types of synthetic fluids tested, the polyalkylene glycol derivatives (UCON products) appear to warrant further investigation. Although the polyalkylene glycol derivatives are not as stable as the silicones,

they are much better lubricating fluids. When the UCON fluids break down at decomposition temperatures, they tend to form soluble fluids or volatile products instead of sludge or varnish. The UCON products are available in a wide range of viscosities in either water-soluble or insoluble form; their toxicity is low.

The mechanical design and the material used in the construction of the dental handpiece will influence to some extent the effectiveness of a lubricant. Differences will exist from one handpiece to another of the same manufacturer. Each handpiece will also vary in its performance due to differences in care and use.

The belt tension is quite critical and influences both speed of the handpiece and temperature rise over the bearings. A speed of 14,800 r.p.m. was achieved with one handpiece with a belt tension which provided a separation of 7.5 cm. between the drive and return sides of the belt with a 200-gram load hung from the middle of the span between the motor and the elbow. The speed decreased to 12,250 r.p.m. when the belt tension was increased to give a 5.0 cm. separation. Table V shows the temperature rise in degrees Fahrenheit over the rear and nose bearing when the Midwest Hi-Speed handpiece is operated with a 100-gram load and no load, and with varying belt tension. The lubricant was Midwest M. O. Lubricant.

TABLE V
EFFECT OF BELT TENSION ON BEARING TEMPERATURE

		BELT TENSION 7.5 CM.		BELT TENSION 5.0 CM.	
		NO LOAD	100 GM.	NO LOAD	100 GM.
		°F. Rise Above Ambient Temperature			
1 min.	Rear Bearing	12	12	9	10
	Nose Bearing	22	23	53	32
2 min.	Rear Bearing	18	20	19	19
	Nose Bearing	30	34	65	29
3 min.	Rear Bearing	20	23	24	26
	Nose Bearing	30	43	72	38
4 min.	Rear Bearing	25	27	32	30
	Nose Bearing	26	43	75	51
5 min.	Rear Bearing	25	29	34	32
	Nose Bearing	27	44	81	59

It was observed that when a handpiece was placed into a sterilizing fluid and the excess oil drained off leaving the residual oil as the lubricant, the temperature rise of the bearings was somewhat greater than when the cleaned handpiece was lightly lubricated with an applicator. The greater the amount of residual oil removed from the handpiece, the less was the temperature rise of the bearings. This occurrence might substantiate the common thought that excess lubricant is detrimental. Difficulties resulting from overlubrication far outweigh those arising from underlubrication; excessive heat generation, rapid deterioration of the lubricant and leakage from the instrument will result.[2] Since viscosity is a measure of a lubricant's internal friction, it would appear that the more viscous a lubricant and the greater the amount of lubricant, the more shear forces would occur between the molecule of the fluid. Thus, a greater quantity of heat would be given off.

SUMMARY

This report has presented an evaluation of a representative group of lubricants for the dental handpiece and contra-angle. The viscosities of the liquid petroleum lubricants range from very light oils to heavy oils at 70° F., but become much less widely dispersed as their temperature approaches the operating temperature of the bearings. The greases react as a medium viscosity oil.

The fluids for use as sterilization media, thus far investigated, are either not stable or are of too great viscosity to provide good lubrication. A few of these synthetic fluids have some favorable characteristics and should be investigated more thoroughly.

Some factors to consider in the selection of a proper cleaner for the handpiece and contra-angle are discussed. The effect of belt tension on the speed of the handpiece and operating temperature of the bearings is pointed out.

It appears that from the evidence presented in this report, certain suggestions could be made for the proper day-to-day care of the handpieces and contra-angles, which are to be used at speeds up to 15,500 r.p.m. For operating speeds in excess of 15,500 r.p.m. further investigation should be accomplished.

1. Stoddard Solvent readily removes the petroleum and synthetic lubricants used in this study from the disassembled handpiece and contra-angle. This solvent is of relatively low toxicity, and with reasonable precautions it should present no great fire hazard. Ethyl alcohol might profitably be used as a secondary cleaner to remove moisture from gears and bearings.

2. Highly refined oils of a viscosity less than 150 Saybolt Universal Seconds appeared to provide acceptable lubrication in the 3 handpieces tested when applied sparingly to the disassembled instrument. Molybdenum disulfide appears to lubricate stainless steel on Pyrex sleeve bearings when used according to manufacturer's instructions.

3. The light oils provide adequate lubrication for the gears and bearings of 3 contra-angles tested when sparingly applied directly to these parts.

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